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SPECIAL TOPICS
Environmental fate of herbicides

RESEARCH ARTICLE

Persistence of S-metolachlor in the soil as affected by moisture content

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Abstract: Background: Several factors may affect herbicide fate in the soil, including soil moisture which can affect herbicide availability and degradation and mixture with other degradable herbicides. **Objective:** The objectives of this research were to evaluate the effects of soil moisture content and association with glyphosate on *S*-metolachlor persistence in lowland soil. **Methods:** Greenhouse experiments were conducted in 2011 and repeated in 2012 using a randomized complete block design in a factorial arrangement (3x3x5) with four replications. Factor A included three soil moisture contents (air-dried, water holding capacity, and saturated), and factor B included two herbicide combinations (*S*-metolachlor; *S*-metolachlor + glyphosate) plus an untreated check. Factor C included five intervals between application and sowing of the bioindicator species (rice): 150, 120, 90, 60, and 30 days before sowing. Herbicide injury, height in rice plants, and herbicide concentration in soil was evaluated. **Results:** *S*-metolachlor concentration and injury to rice were higher under dry soil conditions regardless of application timing. In contrast, rice injury was significantly lower in the soil saturated condition. The association with glyphosate did not affect *S*-metolachlor persistence in lowland soil. **Conclusion:** These results indicate that *S*-metolachlor exhibits shorter persistence under saturated soil conditions; and indicated that drought periods following *S*-metolachlor applications may increase carryover to sensitive crops planted in rotation.

Keywords: Carryover; Chloroacetamide; Crop Rotation; Rice Paddy

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1. Introduction

Crop rotation is an important tool for integrated weedy rice management in paddy fields (Avila et al., 2021) and soybeans is a good alternative in these areas (Junkes et al., 2022). Among the herbicides used in soybeans *S*-metolachlor is an selective herbicide (Adegas et al., 2022) and efficient to control weedy rice in pre-emergence or early-post emergence in tank mixture with glyphosate (Zemolin et al., 2014; Bertucci et al., 2019).

S-metolachlor is a nonionic herbicide with residual activity in soil, controlling annual grasses and small-seeded broadleaf weeds and can be applied in preemergence (PRE), or early postemergence (EPOST) of tolerant crops (Szarka et al., 2022). Its mode of action is based on the inhibition of very-long-chain fatty acids, interrupting shoot development in susceptible plants (Hwang et al., 2023). *S*-metolachlor has moderate water solubility (480 mg L^{-1} at 25°C) and soil organic carbon sorption coefficient (K_{oc}) of 200 ml g^{-1} with adsorption positively correlated with organic matter and clay content (Shaner, 2014).

S-metolachlor's half-life in soil ranges from 2.5 to 289 days (Rice et al., 2002). Thus, there is a concern that *S*-metolachlor may carryover to sensitive crops, including rice planted in rotation. Herbicide persistence and carryover in soil may be affected by several factors such as physicochemical properties of molecule, soil characteristics, environmental conditions (Price, Price, 2020), association between herbicides (Nunes and Vidal, 2008) or even interactions between these factors. Among soil characteristics, moisture content influences herbicide availability in the soil solution, microbial activity, and other dissipation processes (Gehrke et al., 2021). For example, under drought conditions, herbicide concentration in the soil solution is reduced due to an increase in adsorption to the soil particles reducing herbicide availability to biodegradation. In contrast, microbial activity can be impaired under low O_2 availability such as flooded conditions (Gehrke et al., 2021).

The persistence of residual herbicides in soil may be also affected by association with glyphosate. This hypothesis has been proposed that some microorganism use glyphosate as an additional nutrient source, increasing microbial biomass and activity, which typically results in greater mineralization of the residual herbicide (Nunes, Vidal, 2008). Laboratory experiments showed that the association of fluometuron with glyphosate increased fluometuron mineralization and dissipation compared to fluometuron applied alone (Lancaster et al., 2008).

Several studies have reported *S*-metolachlor carryover and persistence in aerobic conditions. However, limited information is available concerning its persistence in poor drained areas such as lowland rice soils. Moreover, additional research is needed to determine whether glyphosate influences *S*-metolachlor dissipation in soil. Therefore, this study was designed to evaluate the effects of moisture content and association with glyphosate on *S*-metolachlor dissipation in lowland soil.

2. Material and Methods

The study was carried out in greenhouse conditions at the Weed Science Research Group (CEHERB), at Universidade Federal de Pelotas, Capão do Leão, Rio Grande do Sul State, Brazil (31°48’03”S 52°24’40”W). The experimental design was a randomized complete block in a factorial arrangement with four replications. Experiments were conducted in 2011 and repeated in 2012. Factor A included three soil moisture contents: air-dried (-100 kPa), water holding capacity (-33 kPa) and saturated conditions (0 kPa). Factor B were herbicide treatments: *S*-metolachlor at 1920 g a.i. ha^{-1} (Dual Gold™, 960 g L^{-1}); *S*-metolachlor at 1920 g a.i. ha^{-1} + glyphosate at 1860 g a.e. ha^{-1} (Roundup Original DI™, 370 g L^{-1}, diammonium salt); and an untreated check. Factor C included five herbicide application timings (150, 120, 90, 60, and 30 days before rice sowing (DBS), which was used as a bioindicator crop). The rice crop was chosen as a bioindicator crop because it is a plant sensitive to S-metolachlor (Zhang et al., 2000). Soil water-holding capacity was determined using the chamber pressure method (Richards, 1965) and moisture content was kept during the whole experiment by weighting each pot daily and replacing the amount of water needed to keep the soil moisture at the desired level. Herbicide rates used in the experiment were defined according to the labeled rate.

The soil used in the experiment was a sandy-loam collected from a rice paddy field without herbicide application for the past five growing seasons. Soil physicochemical properties are presented in Table 1. The experimental units were 2.0 L plastic pots filled with 1.2 kg of air-dried soil previously sieved in a 2 mm-mesh sieve. Prior to implementing the soil-moisture, moisture content was determined, and it was discounted from the total needed to reach the desired moisture content for each treatment. Herbicide were applied using a CO_2-backpack sprayer coupled to a three-nozzle boom (Teejet XR11002) spaced at 50 cm and calibrated to deliver 150 L ha^{-1} of spray solution at 172 kPa.

Moisture contents were adjusted immediately after herbicides applications and monitored daily by weighing the pots during the experimental period (Figure 1). Also, monthly average air temperature was recorded (Figure 2). The bioassay was performed using the rice variety IRGA 417 after herbicide application in the intervals of each treatment.

Table 1 - Physicochemical properties of soil used in the experimental

pH water	Clay	Sand	Silt	O.M	Phosphorus	Potassium	Calcium	Magnesium	Aluminum
(1:1)	(%)				mg dm^{-3}			cmol$_c$ dm^{-3}	
5.2	16	52	32	1.3	6.7	43	4.3	2.0	0.1

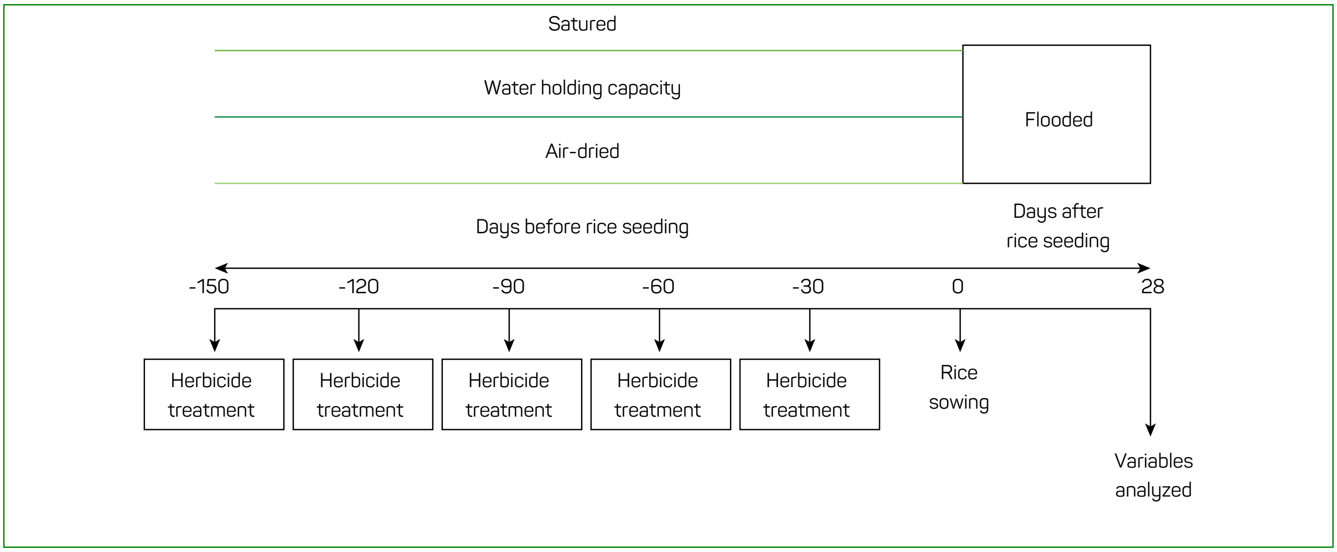

Figure 1 - Experiment scheme on cultivar IRGA 417 during 2011 and repeated in 2012

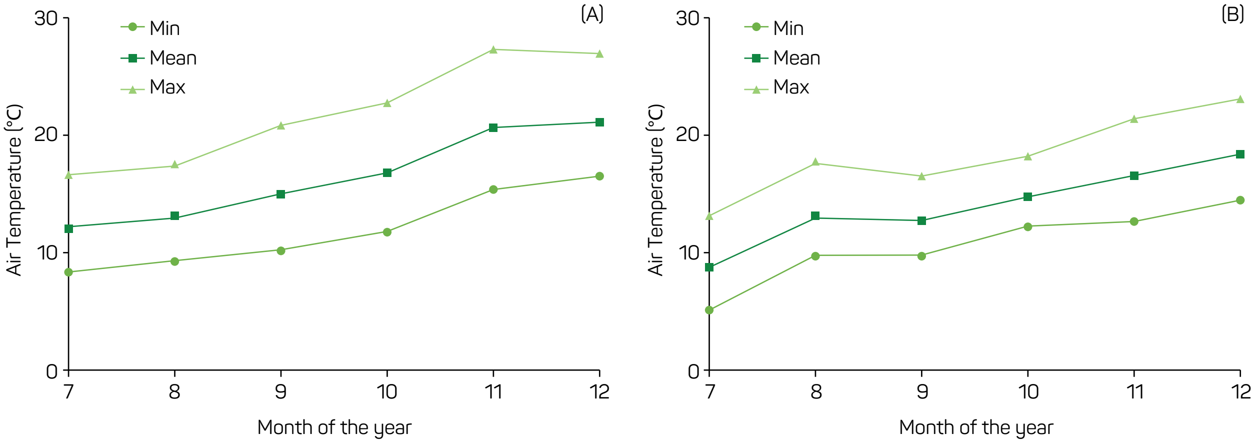

Figure 2 - Monthly air temperature (maximum, mean and minimum) recorded from July to December in 2011 (A) and 2012 (B)

Rice sowing was performed under a pre-germinated system. Soil was flooded one day prior to rice planting to standardize the moisture content between treatments.

Herbicide injury to rice plants was estimated visually by grading from 0 to 100% where 0 = no herbicide injury symptoms and 100 = death of the rice plants (Sociedade Brasileira da Ciência de Plantas Daninhas, 1995). Plant height was determined by measuring the length (cm) from the soil surface to the flag-leaf tip. Shoot dry matter was obtained by harvesting the rice plants near the soil surface. All the evaluations were performed at 28 days after the bioindicator crop establishment (DAE). Data were expressed based on a percent of untreated-check.

In 2011, soil samples were collected after 28 DAE to determine the herbicide concentration (mg kg^{-1}) remaining in soil. Samples were analyzed by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) at the Laboratory of Pesticide Residues Analysis (LARP) at the Universidade Federal de Santa Maria. The UHPLC-MS/MS system from Waters (USA) was equipped with Acquity binary pump and MS triple quadrupole Xevo TQ with electrospray ionization (ESI) source, opered in selected reaction monitoring (SRM) mode. Neat pesticide standards of the compounds in study were from LGC (Wesel, Germany) with purity greater than 98%. Sample preparation was based on the method described by Martins et al. (2014).

Data were tested for the assumptions of ANOVA, testing normality Shapiro Wilk test, homogeneity using Hartley's test and error independence checked graphically, were compared using statistical program R Core Team using ExpDes.pt package (Ferreira et al., 2014). Because of a lack of homogeneity of variances between data sets, data were not combined and are presented separately by years. All variables evaluated were transformed to root square equation $\sqrt{(x+0.5)}$ and then subjected to ANOVA ($p \leq 0.05$). Ninety-five percentage confidence intervals were used to compare means between treatments. The graphics were performed using PRISM (GraphPad Software™, Boston, MA).

3. Results and Discussion

The association with glyphosate did not affect *S*-metolachlor persistence in this study (data not presented). However, an interaction between soil moisture content and application timing was observed for all variables evaluated. Greater herbicide injury occurred in dry soil condition with no rice establishment observed regardless of *S*-metolachlor application timing (Figure 3), demonstrating that even application of *S*-metolachlor 150 days prior to rice sowing did affect rice establishment. In 2011, under water holding capacity conditions, rice establishment was observed in the treatment at 90 days before rice seeding (DBS), however, injury was still similar to the injury observed in plants under dry soil condition. The lowest injuries were observed in the treatment with saturated soil from 90, 120, and 150 DBS (Figure 3A). However, the levels of injury were close to 80%.

In 2012, there were differences in treatments under water holding capacity and soil saturated (Figure 3B). In the water holding capacity treatment, the levels of injury in the rice plants reduced as the time of herbicide application increased, being in the times 90, 120, and 150 DBS, with 32, 30 and 42%, respectively. In saturated soil, rice plants had the lowest injuries, presenting 59% injury at 60 DBS, and showing no injuries at 90, 120, and 150 DBS. In general, herbicide injury in water holding capacity and saturated soil were lower in 2012 than 2011 (Figures 3A, and 3B).

Results have shown that under field conditions, in the application of *S*-metolachlor in the fall-winter season, with saturated soil condition, weedy-rice injury of 44 and 84% was verified in the 2017 and 2018 growing seasons, respectively (Bertucci et al., 2019). The residual period of *S*-metolachlor was 149 days in 2016–2017 and 155 days in 2018–19 in

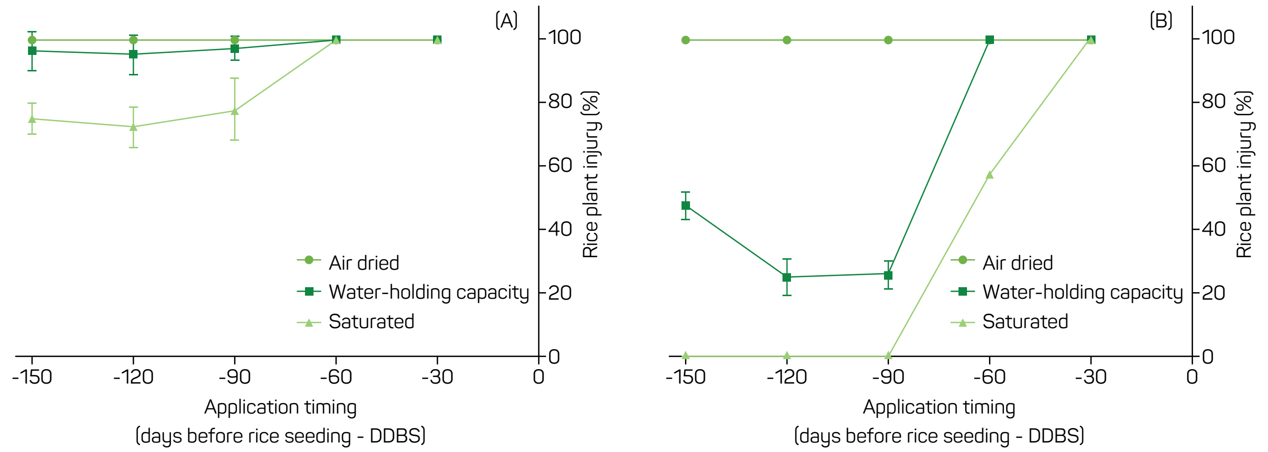

Figure 3 - Rice injury (%) at 28 days after plant establishment under three soil moisture contents (air-dried, water holding capacity and satured) and five application timings of *S*-metolachlor (150, 120, 90, 60, and 30 days before rice seeding) in season 2011 (A) and 2012 (B). Error bars represent 95% confidence intervals of four replications (n=4)

saturated soil conditions, demonstrating carryover effect of *S*-metolachlor in this soil condition (Bertucci et al., 2019). In another study of field conditions, injury of rice plants from 9 to 30% was verified in the 2010–11 and 2011–12 season (Lawrence et al., 2018). In this study, the residual period of *S*-metolachlor was 185 and 190 days (2010-11 and 2011–12 seasons, respectively), and soil moisture was close to the water holding capacity condition (Lawrence et al., 2018). The data corroborate the results seen in this study in 2017 (Figure 3A), and demonstrate that there is variability in the soil herbicide residual, similar to the results of 2018 (Figure 3B). Environmental effects, such as temperature, may explain the difference found between the 2017 and 2018 harvests, where the average temperature was 16.4 and 18.3 °C (Figure 2).

Regarding plant height, there was no plant emergence at 30 DBS (2011 and 2012) and 60 DBS (2011) for all treatments (Figure 4). The behavior of plant height was similar to the injury variable, with the water holding capacity. Treatment presenting the smallest heights at 90, 120, and 150 DBS in relation to the treatment with saturated soil. In 2012, the plants in the treatment in saturated soil, presented heights like the treatments without herbicide at 90, 120, and 150 DBS. Corroborating with the results of this work, it was verified that the application of *S*-metolachlor (1420 or 2840 g a.i. ha^{-1}) at 185 – 190 days after application in conditions of soil moisture close to water holding capacity, led to a reduction in height of 10% rice plants compared to non-treated plants (Lawrence et al., 2018). This result was similar to those found in the 2012 season (Figure 4B), wherein the water holding capacity and soil saturated treatments, the reduction in height was approximately 10% at 90, 120, and 150 DBS.

S-metolachlor persistence was shorter in soil with greater moisture content (Figure 5). The concentration of *S*-metolachlor in drained soil did not vary over time, with an average concentration of 0.2 mg kg^{-1}. These values corroborate with the results visualized in the other variables (Figure 3, and 4), where there was no emergence of rice plants. Under water holding capacity conditions, there is a reduction in *S*-metolachlor concentration levels, with an average concentration of 0.093 mg kg^{-1} in the periods of 30, 60, and 90 DBS, and an average concentration of 0.02 mg kg^{-1} at 120 and 150 DBS. The levels of the *S*-metolachlor herbicide explain the other analyzed variables (Figure 3-5), where there is no emergence of plants at 30 and 60 DBS, demonstrating that this herbicide concentration is still toxic for rice plants. Already at 120, and 150 DBS in the water holding capacity condition, despite the *S*-metolachlor concentration, there is already plant establishment.

In saturated soil conditions, the lowest concentrations of *S*-metolachlor were verified in relation to the other soil moisture treatments. At 30 and 60 DBS the average concentration of *S*-metolachlor was 0.05 mg kg^{-1}, an herbicide concentration sufficient to inhibit the emergence of rice plants (Figure 3). At 90, 120, and 150 DBS the concentration of *S*-metolachlor was 0.02 mg kg^{-1}, a condition that allowed the establishment of plants, but with an injury level still close to 80%.

As *S*-metolachlor has moderate water solubility, the greater moisture content in saturated soil may have contributed to enhance *S*-metolachlor availability to microbial degradation and other dissipation processes. Research found that halogenated chemicals, such as *S*-metolachlor might be degraded by reductive dehalogenation under low oxygen conditions, involving a cometabolic process responsible to disrupt the C-Cl binding (Scheunert et al., 1992). As a rule, the products of this reaction exhibit lower toxicity and are easily degraded (Mikesell, Boyd, 1986).

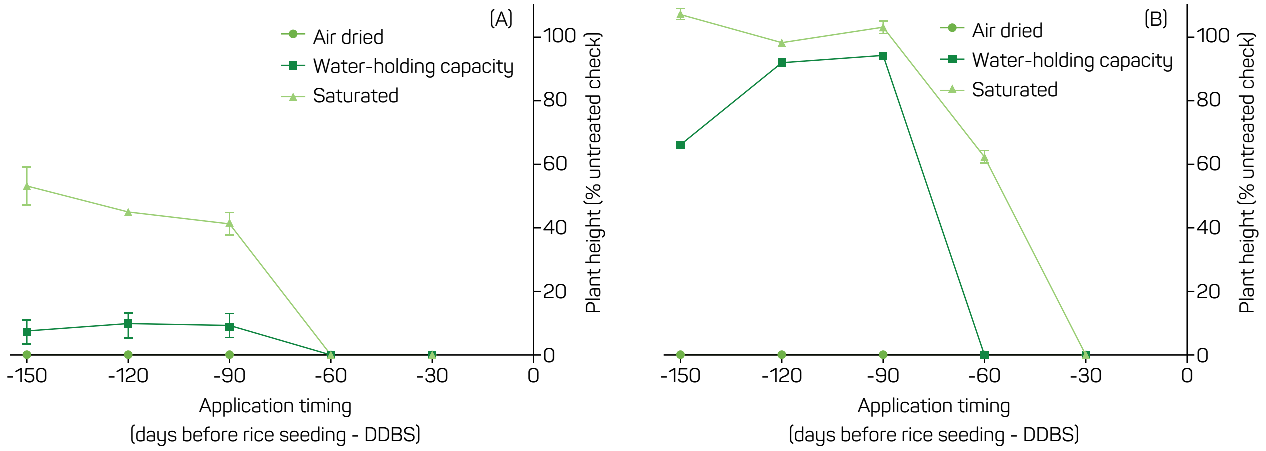

Figure 4 - Rice plant height (%) at 28 days after plant establishment under three soil moisture contents (air-dried, water holding capacity and satured) and five application timing of *S*-metolachlor (150, 120, 90, 60, and 30 days before rice seeding) in season 2011 (A) and 2012 (B). Error bars represent 95% confidence intervals of four replications (n=4)

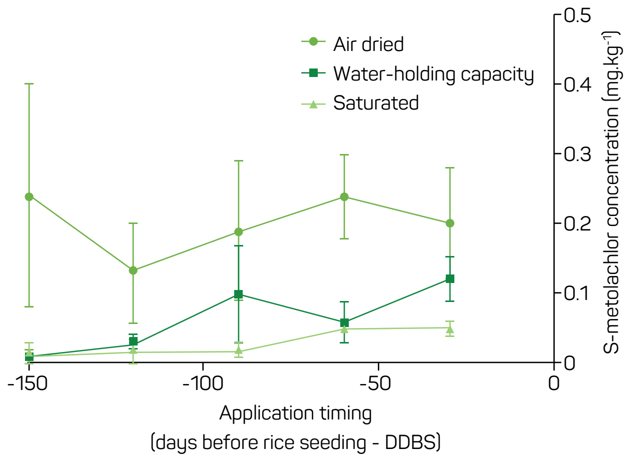

Figure 5 - *S*-metolachlor concentration (mg kg^{-1}) in lowland soil collected 28 days after plant establishment under three soil moisture contents (air-dried, water holding capacity and satured) and five application timings of *S*-metolachlor (150, 120, 90, 60, and 30 days before rice seeding) in season 2011. Error bars represent 95% confidence intervals of four replications (n=4)

The formation of bound residues of herbicide in soil might occur after the reductive dehalogenation process. In general, the formation rates of bound residues increase as the chloride content in the molecule decreases (Scheunert et al., 1985). Evaluating *S*-metolachlor degradation and persistence, (Rice et al., 2002) reported higher bound residues formation in saturated soil. Similar results were observed to dimethenamid herbicide in anaerobic condition, which approximately 50% was incorporated to the soil under bound residues form (Crawford et al., 2002).

Soil moisture influences the residual effect of *S*-metolachlor in the soil and consequently its carryover effect on rice plants. In saturated soil conditions in the field, the establishment of weedy rice plants was 58% in relation to nontreated plants, while in the field condition of soil without irrigation it was 24% in relation to nontreated plants with *S*-metolachlor (1,440 g ai ha^{-1}) (Oliveira Neto et al., 2020).

S-metolachlor injury to rice was higher in the dry soil conditions. A reduction in soil moisture content causes an increase in herbicide adsorption, decreasing its availability to biodegradation (Christoffoleti et al., 2008). In addition, microbial activity may be reduced or even impaired in soils with low moisture content since microorganisms need water to keep their vital functions. In contrast, when water was added to the experimental units to standardize the soil moisture content and allow rice sowing, the herbicide adsorbed to the soil might have been desorbed to the soil solution, increasing injury potential to rice.

According to rice injury results, *S*-metolachlor persistence in soil was shorter in 2012 than 2011. Rice recovery started at 60 DBS in 2012 instead of 90 DBS in 2011. This reduction may have resulted from a faster degradation process caused by higher temperatures observed in 2012 compared to 2011 (Figure 2). Higher temperatures increase cyanobacteria and bacteria activity enhancing the ability to degrade chloride compounds, such as *S*-metolachlor. Thus, a stimulus on soil microorganism population might have contributed to faster dissipation and consequently shorter *S*-metolachlor persistence in 2012.

4. Conclusions

S-metolachlor exhibits shorter persistence under saturated soil conditions and it has no risk of carryover under these conditions. But under dry conditions, *S*-metolachlor showed to be very persistence with low degradation in the 150 days of the experiment. These results show us that during regular years with regular precipitation

S-metolachlor carryover may not occur, but in years with low precipitation, with dry periods after *S*-metolachlor applications may increase herbicide persistence and the risk of carryover to sensitive crops planted in rotation.

Author's contributions

All authors read and agreed to the published version of the manuscript. CRZ, ERC, and LAA: conceptualization of the manuscript and development of the methodology. CRZ, GVC, LLC, APVC, and RZ: data collection and curation. CRZ, GVC, LLC, and APVC: data analysis. CRZ, MVF, RZ, ERC, and LAA: data interpretation. ERC, and LAA: funding acquisition and resources. LAA: supervision. CRZ, and MVF: writing the original draft of the manuscript. CRZ, MVF, ERC, and LAA: writing, review and editing.

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