UNIVERSIDADE DE SÃO PAULO

CENTRO DE ENERGIA NUCLEAR NA AGRICULTURA

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Environmental fate and behavior of mesotrione alone and mixed with S-metolachlor and terbuthylazine in Brazilian soils

Piracicaba

2017

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Tese apresentada ao Centro de Energia Nuclear da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Área de Concentração: Química na Agricultura e no Ambiente

Orientador: Prof. Dr. Valdemar Luiz Tornisielo

Piracicaba

2017

Dados Internacionais de Catalogação na Publicação (CIP)

Seção Técnica de Biblioteca - CENA/USP

Mendes, Kassio Ferreira
Destino ambiental e comportamento do mesotrione isolado e misturado com S-metolachlor e terbuthylazine em solos brasileiros / Environmental fate and behavior of mesotrione alone and mixed with S-metolachlor and terbuthylazine in Brazilian soils / Kassio Ferreira Mendes; orientador Valdemar Luiz Tornisielo. - - Piracicaba, 2017.
133 p. : il.

Tese (Doutorado – Programa de Pós-Graduação em Ciências. Área de Concentração: Química na Agricultura e no Ambiente) – Centro de Energia Nuclear na Agricultura da Universidade de São Paulo.

1. Controle químico 2. Herbicidas 3. Impactos ambientais 4. Plantas daninhas 5. Poluição do solo 6. Resíduos de pesticidas em solos 7. Solo tropical I. Título

CDU 632.95.028 : 632.954

To my parents, Aparecido and Mércia, for their support and encouragement.

I offer and dedicate

ACKNOWLEDGMENTS

First and foremost, I would like to thank God, whose many blessings have made me who I am today. You have given me the power to believe in myself and pursue my dreams. I could never have done this without the faith I have in you, the Almighty.

I am forever grateful to Dr. Valdemar Luiz Tornisielo for having accepted to be my thesis advisor and for giving me a good opportunity to work in his group at Ecotoxicology Lab. His patience, guidance, charisma and effort were essential to the birth of this document and to my formation as a future researcher. His constant feedback on technical aspects skills helped me in honing my knowledge.

I express my profound gratitude to Center of Nuclear Energy in Agriculture of Univerisy of São Paulo which helped me develop my skills.

I am really grateful to Rodrigo Floriano Pimpinato and Carlos Alberto Dorelli for all the help he provided throughout my research. I appreciate all their contributions of time and ideas to make my Ph.D. experience productive and stimulating. I would like to take this opportunity to express my deepest gratitude to Renata Pousa Maziero for all the the contribution and attention.

My sincere thanks to the members of my research group formed by Rosana Maria de Oliveira Freguglia, Ana Carolina Dias Guimarães, Graziela Cristina Rossi Moura Andrade, Jeane Gisele Francisco, Rafael Grossi Botelho, Fabrícia Cristina dos Reis, Bianca Assis Barbosa Martins, Franz Zirena Vilca, Leila Aparecida Figueiredo, Marcela Lembi Viti, and Lucineide Aparecida Maranho have contributed immensely to my personal and professional time at CENA. The group has been a source of friendships as well as good advice and collaboration.

I am especially grateful for my ex-advisors Dr. Miriam Hiroko Inoue (undergraduation) of University of Mato Grosso State, Dr. Marcelo Rodrigues dos Reis (graduation - Master) of University of Federal Viçosa, Dr. Kurt Spokas (BEPE, graduation – Ph.D.) of University of Minnesota and United States Department of Agriculture.

I also thankful my parents, Aparecido Ferreira Mendes and Mércia Ferreira da Silva, who raised me with a love of science and supported me in all my pursuits. I also extend my thanks to my sisters Keyla Ferreira Mendes and Karla Ferreira Mendes; and my nephew Kauã Mendes Costa who have supported me throughout my academic trajectory. I am also grateful to all my friends whose encouraging words kept me stimulated.

On a different note I would like to thank the São Paulo Research Foundation (FAPESP) [process 2013/25399-3 and BEPE - process 2015/16655-1 (Appendix - full text)] for the financial support throughout my Ph.D. in Science Program.

Finally, I express my sincere thanks my loving, supportive, encouraging, and patient wife Andreia Correia da Silva whose faithful support during this Ph.D. is so appreciated. The joy and enthusiasm she has was contagious and motivational for me, even during tough times in the Ph.D. pursuit.

There are so many others whom I may have inadvertently left out and I sincerely thank all of them for their help. Thank you very much!

"I will put among scholars on Earth, I will give you
the title of Doctor and I will put a
Dr. in your name.
Amen!"
Unknown author

ABSTRACT

MENDES, K. F. **Environmental fate and behavior of mesotrione alone and mixed with S-metolachlor and terbuthylazine in Brazilian soils.** 2017. 133 p. Tese (Doutorado) - Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2017.

The mixture of herbicides is a widely used technique in weed control in several crops, including maize. However, interactions that might potentially result from mixing herbicides are a matter of constant concern and research. Soils are ideal settings to study transport and behavior of herbicides along gradients of physicochemical properties. The aim of this study was to evaluate sorption-desorption, leaching mineralization, biodegradation, and microbial respiration of mesotrione applied alone and mixed with S-metolachlor + terbuthylazine in samples from Brazilian sites cultivated with maize. The sorption-desorption of ^{14}C-labeled [cyclohexane-2-^{14}C] mesotrione was evaluated using the batch method, leaching was in glass columns, mineralization and degradation experiments were conducted using biometric flask, and microbial respiration was established according to the soil microorganisms: carbon transformation test with ^{14}C-glucose solution in biometric flasks as well. Sorption of mesotrione applied alone and mixed had K_d sorption coefficient) values ranging from 0.08 to 5.05 kg L^{-1} and from 0.09 to 5.20 kg L^{-1}, respectively, with similar behavior across soils. Mesotrione sorption was influenced primarily by the clay mineral (CM) content and the soil pH. Leaching of mesotrione is relatively high in the tropical soils and correlates with the pH (R^2 = -0.84) and CM content (R^2 = 0.75) and may pose a potential groundwater contamination risk. From the 49 d laboratory incubation data, increased mineralization half-life of mesotrione were observed for the mixture of herbicides, ranging from a 4 d increase for the sandy loam soil to a 1 d increase in the sandy clay texture soils. Mesotrione degradation rate had a 2-fold increase in the sandy loam compared to the sandy clay soil. Two metabolites can be identified from mesotrione degradation, 4-methyl-sulfonyl-2-nitrobenzoic acid (MNBA) and 2-amino-4-methylsulfonyl benzoic acid (AMBA). For unamended soil – control (without herbicide), microbial activity followed similar behavior to amended soil with herbicides in total $^{14}CO_2$ released and accumulated, ranging from 23 to 27%. The mode of application of mesotrione did not influence sorption, desorption or leaching. Mesotrione sorption was relatively low in soils, which creates the high potential for leaching in maize producing areas. Thus, recommendations for mesotrione application, without the prior knowledge of the soil physical and chemical properties can result in an inefficient weed control. Mesotrione biotransformation was relatively quick, indicating this herbicide has low persistence, and consequently, low residual effect on crops and weeds when present in similar soils to this present study. Microbial respiration for all treatments was slightly higher in the sandy clay compared with sandy loam soil; although soil samples with application of herbicides (alones and in a mixture) did not have decreased basal microbial respiration or mineralization rate of glucose.

Keywords: Herbicide mixture. Transport. Retention. Transformation. Tropical soils.

RESUMO

MENDES, K. F. **Destino ambiental e comportamento do mesotrione isolado e misturado com S-metolachlor e terbuthylazine em solos brasileiros.** 2017. 133 p. Tese (Doutorado) - Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2017.

A mistura de herbicidas é uma técnica amplamente utilizada no controle de plantas daninhas em diversas culturas, incluindo o milho. No entanto, as interações que podem potencialmente resultar da mistura de herbicidas são uma questão de constante preocupação e pesquisa. Os solos são os locais ideais para estudar o transporte e o comportamento de herbicidas ao longo dos gradientes de propriedades físico-químicas. O objetivo deste estudo foi avaliar a sorção-dessorção, lixiviação, mineralização, biodegradação e respiração microbiana do mesotrione aplicado isolado e misturado com S-metolachlor + terbuthylazine em amostras de locais brasileiros cultivados com milho. A sorção-dessorção do mesotrione ^{14}C-marcado [ciclohexano-2-^{14}C] foi avaliada utilizando o método de lote, a lixiviação foi em colunas de vidro, experimentos de mineralização e de degradação foi realizados utilizando frascos biométricos e a respiração microbiana foi estabelecida de acordo com os microorganismos do solo: teste de transformação de carbono com uma solução de ^{14}C-glicose em frascos biométricos também. A sorção do mesotrione aplicado isolado e misturado apresentou valores de K_d (coeficiente de sorção) varando de 0,08 à 5,05 kg L^{-1} e de 0,09 à 5,20 kg L^{-1}, respectivamente, com comportamento semelhante nos solos. A sorção do mesotrione foi influenciada principalmente pelo teor de argila mineral (CM) e o pH do solo. A lixiviação do mesotrione é relativamente elevada nos solos tropicais e correlaciona-se com o pH (R^2 = -0,84) e teor de CM (R^2 = 0,75) e pode representar um potencial risco de contaminação das águas subterrâneas. A partir dos dados de laboratório de 49 dias de incubação, o aumento da meia-vida de mineralização do mesotrione foi observado para a mistura de herbicidas, variando de um aumento de 4 dias do solo franco-arenoso para um aumento de 1 dia nos solos de textura argilo-arenosa. A taxa de degradação do mesotrione apresentou um aumento de 2 vezes no franco-arenoso em relação ao solo de textura argilo-arenosa. Dois metabólitos podem ser identificados a partir de degradação do mesotrione, o ácido 4-metil-sulfonil-2-nitrobenzóico (MNBA) e 2-amino-4-metilsulfonil benzóico (AMBA). Para o solo sem alterações - controle (sem aplicação de herbicida), a atividade microbiana seguiu comportamento similar ao solo tratado com herbicidas no total de $^{14}CO_2$ liberado e acumulado, variando de 23 a 27%. O modo de aplicação do mesotrione não influenciou a sorção, dessorção ou lixiviação. A sorção do mesotrione foi relativamente baixa nos solos, que gera alto potencial de lixiviação em áreas produtoras de milho. Assim, as recomendações para a aplicação do mesotrione, sem o conhecimento prévio das propriedades físicas e químicas do solo podem resultar em um ineficiente controle de plantas daninhas. A biotransformação do mesotrione foi relativamente rápida, indicando que este herbicida tem baixa persistência, e, consequentemente, baixo efeito residual sobre as culturas e plantas daninhas, quando presentes em solos semelhantes ao presente estudo. A respiração microbiana em todos os tratamentos foi ligeiramente superior no argilo-arenoso em comparação com o solo franco-arenoso; embora as amostras de solo com aplicação de herbicidas (isolados e em uma mistura) não têm diminuído a respiração microbiana basal ou a taxa de mineralização da glicose.

Palavras-chave: Mistura de herbicidas. Transporte. Retenção. Transformação. Solos tropicais.

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SUMMARY

1. INTRODUCTION

Effective monitoring the weed control is a major obstacle to the maize production (VISSOH et al., 2004), where the average total income losses due to competition from weeds can vary between 34 and 60% (OERKE, 2006). Therefore, the competitiveness of maize against weeds is very low, especially in the early stages of growth (SWANTON; WEISE, 1991).

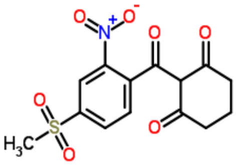
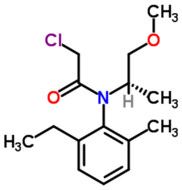
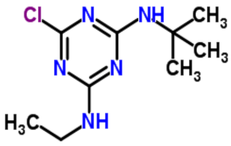
Among the factors influencing the interference highlights the period in which the weed population is competing for environmental resources with the culture in which the use of control measures necessary to reduce the negative effects of interference (SILVA et al., 2007a). Among these, chemical control has been highlighted by the efficiency in weed control, speed of operation and cost savings when compared to other methods. However, the effectiveness of herbicide varies among them, depending of environmental conditions, application time and weed species to be controlled (MEROTTO JUNIOR et al., 1997).

Therefore, when herbicides are applied to the soil in pre-emergence, they are exposed to environmental conditions and may be degraded by the influence of physical, chemical and biological agents, volatilized, adsorbed by soil colloids determining the residual effect, desorbed, and transported externally by runoff and leaching (SILVA et al., 2007b; WESTRA et al., 2014).

Blasioli et al. (2011) reported that the prediction of the movement and the fate of herbicides in soils represent an important strategy in limiting their environmental impact. The chemico-physical properties of herbicides affect their behavior in soil and regulate their interaction mechanisms with organic and inorganic soil phases. Among these, dissolved organic matter (DOM) plays an important role: DOM influences the mobility of herbicides by complex interactions that can facilitate or reduce the movement of chemicals along the soil profile. The knowledge of soil phase characteristics and the mechanisms involved in herbicide transformation can help to understand the fate of herbicides in soil.

Mesotrione [2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione], S-metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl] acetamide], and terbuthylazine [6-chloro-*N*-(1,1-dimethylethyl)-*N*'-ethyl-1,3,5-triazine-2,4-diamine] are among the most used herbicide for maize, for control several weed in pre- and early postemergence within Brazil. Their vast application and physicochemical properties (Table 1.1).

Table 1.1 - Structural formulas and physical and chemical attributes of herbicides.

Attribute	mesotrione	S-metolachlor	Terbuthylazine
Structural formula			
Molecular formula	$C_{14}H_{13}NO_7S$	$C_{15}H_{22}ClNO_2$	$C_9H_{16}ClN_5$
Chemical group	Triketones-F2	Chloroacetamides-K3	Triazines-C1
Molecular weight (g mol^{-1})	339.32	283.79	229.71
Solubility in water at 20°C (mg L^{-1})	160	480	6.6
Log K_{ow}	0.11	3.05	6.60
pKa at 25°C	3.12	Not ionizable	1.90
Vapor pressure at 25°C (Pa)	5.69×10^{-6}	3.70×10^{-3}	0.12×10^{-3}
DT50 (days)	32	15	75.1
K_d (L kg^{-1})	1.62	1.88	5.1
K_{oc} (L kg^{-1})	122	226.1	231

Source: Adapted from PPDB (2017).

Mesotrione is a herbicide registered for use in pre- and postemergence of broadleaf weeds in maize. This herbicide inhibits carotenoid, 4-hydroxyphenyl-pyruvate dioxygenase enzyme (HPPD), which converts tyrosine to plastoquinone and α-tocopherol, producing symptoms bleaching the leaves (MITCHELL et al., 2001).

S-metolachlor herbicide is a non-ionizable compound, which belongs to the chemical group of the chloroacetamide, which inhibits protein synthesis and chlorophyll in plants but has a low toxicity to mammals. It is applied in preemergence or preplant incorporated to control some monocotyledons and dicotyledons, selectively for crops of maize and soybeans (AHRENS, 1994).

Terbuthylazine is a member of the triazine family and inhibits photosynthesis by inhibiting electron transfer at the reducing site of photosystem II in the chloroplasts (GOOD, 1961), and is widely used as an alternative for atrazine and as a selective herbicide for vegetation management in agricultural and forest production. However, the mixture formulated mesotrione + S-metolachlor + terbuthylazine provides satisfactory control both in pre- and early postemergence of weeds in maize (RAPPARINI; FABBI, 2005).

Behavioural studies using mixtures of herbicides are fundamental for improving stewardship guidelines. In the agricultural and environmental setting, herbicides are frequently applied in mixture with different herbicides, in varying soil types, and weather conditions. Therefore, the effects of herbicide mixtures on herbicide mineralization, degradation, sorption, desorption, and leaching processes in the soil must be understood in order to make herbicide use more efficient and environmentally sustainable. Given the above, the hypothesis of this thesis is that application of mesotrione alone interferes in environmental fate and behavior of the mixture formulated mesotrione + S-metolachlor + terbuthylazine in soils cultivated with maize.

1.1. Objectives

1.1.1. General objective

The general objective of this thesis was to evaluate environmental fate and behavior of mesotrione alone and mixed with S-metolachlor and terbuthylazine in Brazilian soils cultivated with maize. Results from this study will aid the understanding of key parameters responsible for the behavior of mesotrione in agricultural areas of Brazil and information on fate of these herbicides in the soils is crucial in assessing environmental impact and risk of chemical applications.

1.1.2. Specifics objectives

Evaluate sorption and desorption of mesotrione applied alone and mixed with S-metolachlor and terbuthylazine in seven Brazilian soils from areas cultivated with maize.

Evaluate the leaching of mesotrione alone and in mixture with S-metolachlor and terbuthylazine in seven tropical soils and their correlation with the physical and chemical properties of soils.

Evaluate the mineralization and biodegradation of mesotrione applied alone or in mixture with S-metolachlor and terbuthylazine when applied to two soil types from Brazil.

Evaluate glucose mineralization in soils of contrasting textures from areas cultivated with maize under application of S-metolachlor, terbuthylazine, and mesotrione alone and in a mixture.

2. LITERATURE REVIEW

2.1. Weeds in maize and chemical control

Critical period of interference in maize (*Zea mays* L.) is between growth stages V2 and V7, and weed interference reduced by an average 87% yield grains of control in competition throughout the crop cycle compared to the control without competition with weeds throughout the cycle (KOZLOWSKI, 2002). Regarding the infesting community in Brazil, dicotyledons represented 22.3% of weeds, especially species: *Taraxacum officinale*, *Senecio brasiliensis*, *Rumex obtusifolius* and *Bidens pilosa*, and monocotyledons, 77.7% of the weed community, especially *Brachiaria plantaginea*.

Chenopodium album, *Abutilon theophrasti*, *Taraxacum officinale*, *Ambrosia artemisiifolia* and *Amaranthus* sp. species were the five weed species more abundant in maize cultivated in the US, and were present in 92, 86, 59, 45 and 44% of all fields assessed, respectively, with average densities of 19, 3, 3, 4 and 3 plants m^{-2}, respectively, and grasses and Cyperaceae species were found in 96% of fields with average densities of 25 plants m^{-2} and a height of 7 cm (FICKETT et al., 2013). In the north-central US, Williams et al. (2008) characterized communities of weeds in sweet maize, and the authors found five most abundant weed, and *Panicum dichotomiflorum* Michx., *Setaria faberi* Herrm., *Panicum miliaceum* L., *Chenopodium album* L. and *Abutilon theorphrasti* Medik.

The presence of weeds provided a reduction of 22 and 23% in maize productivity, when the comparison is made between the controls (DAN et al., 2010). It is estimated that in areas with weed control, losses in maize productivity was about 13% (CARVALHO et al., 2007). On the other hand, maize yields without competition with weeds were 2.8 to 3.4 t ha^{-1}. As expected, the performance loss increased with duration of infestation weed, and ranged between 38 and 65% over the free treatment of weeds (GANTOLI et al., 2013).

This flora weed has been traditionally controlled with pre-emergence applications based on mesotrione, S-metolachlor, and terbuthylazine, because of its broad controlled weed spectrum, superior residual activity, excellent crop tolerance, perceivable speed of efficacy, and suitability as partner for other active ingredients (PANNACCI; ONOFRI, 2016). However, short rotation cycles or monoculture of maize with repeated applications of the same pre-emergence herbicides have determined a strong increase in the frequency of several “difficult to control” weed species, forcing farmers to adopt less simplified weed control strategies (MEISSLE et al., 2010).

In particular, in order to optimise weed control efficacy and minimise the application costs, the use of complex combinations of pre and post-emergence herbicides, as well as herbicide mixtures, has become the rule rather than the exception in many countries (KUDSK, 2007; PANNACCI et al., 2007). This strategy also represents an important tool to avoid problems related to herbicide resistance (FRIESEN et al., 2000; NORSWORTHY et al., 2012), but it requires some preliminary information to assist farmers with the process of herbicide and dosage selection, depending on the floristic situation (MATTHEWS, 2008).

2.2. Herbicide mixture

Weed control is intended, among other things, reduce or eliminate weed competition with the crop. It is important to remember that there are hundreds of weed species and that they are present the various morphological and physiological characteristics which give them different behavior (susceptibility, tolerance or resistance) compared to used herbicides. Besides this fact, the need to reduce crop production costs has left producers and manufacturers, to prepare mixtures of herbicides with different active ingredients, or even with other pesticides (SILVA et al., 2007a).

Association of two or more herbicides is a widely used technique in controlling weeds along with other management techniques. Among the advantages presented by association of herbicides is the increased control spectrum (DAMALAS; ELEFTHEROHORINOS, 2001) or increase the control period of weeds (VANGESSEL et al., 2000), reduced costs, lower waste in the environment by the use of lower doses and emergence prevent of weeds resistant to herbicides (GRESSEL, 1990).

Interaction type arising from association is a matter of constant concern and research. Although several classifications adopted to classify these types of interaction, additivity, synergism, and antagonism terms are the most accepted by scientific society and has been used to dose response curves of herbicides and isobolograms to define the type of interaction (VIDAL et al., 2003). Antagonism can occur due to the reduction of absorption (CULPEPPER et al., 1999; BROMMER et al., 2000) or absorption and translocation of graminicides by latifolicide mixtures (HOLSHOUSER; COBLE, 1990; TREZZI et al., 2007). Antagonistic effects mainly occur when the graminicide is applied together or after latifolicide mixtures herbicides.

Although all of these interactions are evaluated herbicide mixture in the plant, little is known on the behavior of mixture in the soil, especially in the sorption-desorption and leaching studies. Herbicide mixture interaction studies are often focused on soil microbiota (JOLY et al., 2014; BAĆMAGA et al., 2015; ŁAWNICZAK et al., 2016). Research in tropical regions, such as Brazil are required to investigation these possible interactions.

There was a great expansion in the use of mixtures and the sequential application of various herbicides in a single crop cycle; however, the management of herbicides, especially mixtures, requires great care, in addition to knowledge about the interactions between products, to obtain maximum weed control and minimize injury to crops (SILVA et al., 2007a). It should be preferred to mixture of commercial products such as mesotrione + S-metolachlor + terbuthylazine, registered as Lumax® in Italy, and used for control of annual grasses and broadleaf weeds in field maize, field seed maize, field silage maize, yellow popcorn, sweet maize and grain sorghum.

2.3. Fate of herbicides in the soil[1]

Knowledge of the environmental fate of herbicides is essential in the evaluation of the risk of environmental hazard. Thus, it is of fundamental importance to know the processes involved in herbicide-soil interaction, aiming to minimize the negative effects on the environment, in particular water resources (BERGSTRÖM et al., 2011; PINHEIRO et al., 2011).

The behavior of the herbicide in the soil profile affects the weeds control period duration and the efficacy of herbicides, especially those applied in pre-emergence, directly into the soil, besides the effects on the environment (WESTRA et al., 2014). Therefore, the study of herbicide behavior has been accomplished through estimates of trends to which they are subject according to

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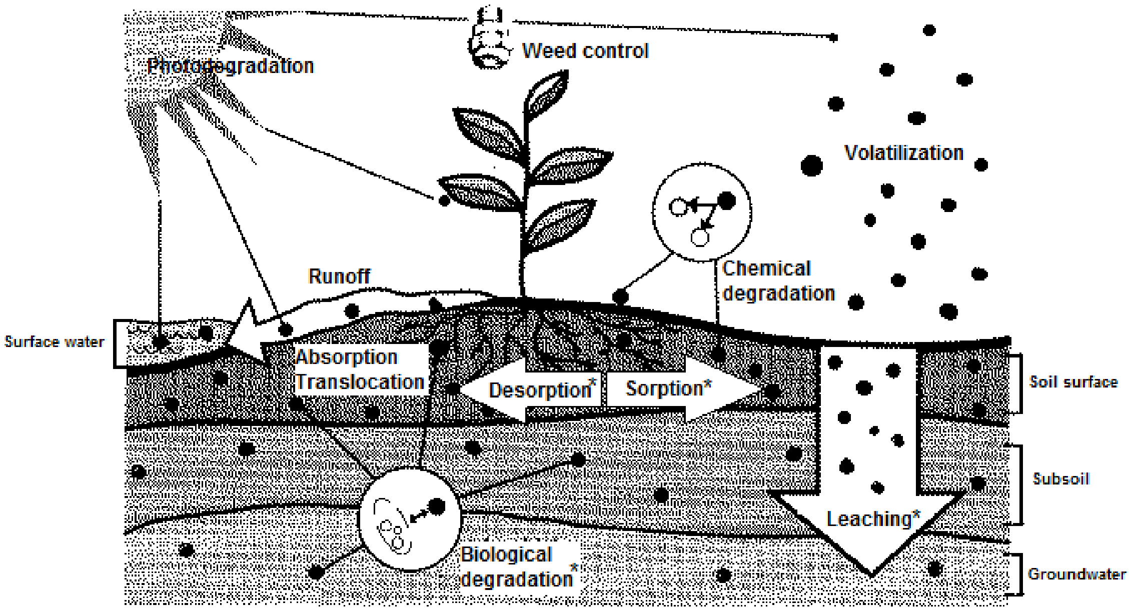
[1] MELO, C.A.D.; DIAS, R.C.; MENDES, K.F.; ASSIS, A.C.L.P.; REIS, M.R. Herbicides carryover in systems cultivated with vegetable crops. **Revista Brasileira de Herbicidas,** Londrina, v.15, n.1, p.67-78, 2016.

[2] MENDES, K.F.; REIS, M.R.; DIAS, A.C.R.; FORMIGA, J.A.; CHRISTOFFOLETI, P.J.; TORNISIELO,

three main processes: retention, transport and transformation (Figure 2.1), which interact with each other, even though these processes are described singly (SILVA et al., 2007b).

Figure 2.1 - Environmental fate and herbicide (●) behavior in the soil. *Experiments were also made in the present study. Source: Adapted from Joern and Lohman (1994).



When a herbicide is applied, several forces can influence its fate (JOERN; LOHMAN, 1994). Ideally it makes contact with the targeted weed. Any remaining herbicide or herbicide residue may be exposed to one, several or all of the pathways shown above.

The retention of herbicides through the soil solid phase theoretically known by sorption is measured by partition coefficients (K_d and K_{oc}) from aqueous solution. Typically, the herbicide sorption increases with the increased content of organic carbon and mineral clay in the soil, thus increasing sorption may retard the movement of the herbicide in the soil (MENDES et al., 2014)[2]. The adsorbed molecules herbicides can return to the soil solution by desorption, or remain retained on an unavailable form, called bound residue (CHRISTOFFOLETI et al., 2008).

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[2] MENDES, K.F.; REIS, M.R.; DIAS, A.C.R.; FORMIGA, J.A.; CHRISTOFFOLETI, P.J.; TORNISIELO, V.L. A proposal to standardize herbicide sorption coefficients in Brazilian tropical soils compared to temperate soils. **Journal of Food, Agriculture and Environment,** Helsinki, v.12, n.3/4, p.424-433, 2014.

The transport is defined as the movement of the herbicide in the soil, which may occur by leaching, runoff, volatilization, absorption and translocation by plants (CHRISTOFFOLETI et al., 2008). The herbicide transport intensity depends on several factors, such as application rate, persistence and mobility, precipitation, topography and local climate. Leaching refers to the vertical movement of the herbicide in the soil depth, the mass flow due to the gravitational force and the water pressure differences in the soil pores (CARTER, 2000), while runoff regards to its lateral movement, on soil surface, both being dependent on rainfall, and the time or intensity of irrigation.

There is evidence that herbicides in the soil tend to be transported quickly to groundwater. The hypotheses proposed to explain this transport include preferential flow, cotransport with colloidal materials and a combination of processes. The rate and magnitude of the rapid transport can be influenced by various parameters such as the physico-chemical properties of herbicide, physico-chemical properties of soil, and soil hydrology and use. For hydrophobic herbicides, for example, their mobility and risk of leaching into groundwaters have been linked to low sorption in soil matrices ($<K_{oc}$ values), emphasizing that higher the sorption of herbicide in the soil, lower is leaching (ESTÉVEZ et al., 2008). Therefore, the non-volatile molecules and water-soluble move in the soil profile following the water flow by water potential difference between two points (PRATA et al., 2003).

Volatilization is the process by which the herbicide is conveyed from the soil to the atmosphere due to the passage of molecules from a liquid to vapor form, depending on its vapor pressure (SILVA et al., 2007b). This is most significant when the residues of herbicides remain on the surface of dry or moist soil, since the incorporation of herbicides in the soil profile can significantly reduce losses caused by volatilization (CARTER, 2000).

The transformation and degradation of the herbicide concerns to changing its molecular structure by biotic and abiotic factors being measured by dissipation time half-life (DT50) - when 50% of the herbicide initially applied is dissipated in soil. The biological degradation, usually carried out by microrganisms and chemical degradation by hydrolysis and oxidation-reduction reactions can be completed, resulting in CO_2, H_2O and minerals (mineralization), or partial, resulting in the formation of metabolites (CHRISTOFFOLETI et al., 2008). The photodecomposition or photolysis is the transformation of the herbicide by sunlight in topsoil.

The period during which an herbicide remains intact and biologically active in the soil is called persistence (BEDMAR; GIANELLI, 2014). Herbicides with greater persistence can result in the phenomenon known as carryover effect, which can be defined as herbicide toxic waste used in the previous crops that remain in the soil, which can affect sensitive crops grown in succession or rotation.

The fate and behavior of herbicides in the soil can be easily estimated by mathematical models (ANDRADE et al., 2011), for the evaluation of soil and water contamination risk; lysimeters installed in the field (GRUNDMANN et al., 2011) and quantification of herbicides by liquid and gas chromatography (MENDES et al., 2016), and studies with radiolabeled molecules - ^{14}C-herbicide, analyzed by liquid scintillation counter, with greater reliability and accuracy the results (MENDES et al., 2017)[3]; besides the use of plant species in the greenhouse, which have a high sensitivity to the herbicide of interest, called bioindicators (INOUE et al., 2002).

2.4. Mesotrione, S-metolachlor, and terbuthylazine behavior in the soil

Mesotrione sorption is dependent on the OC content and pH of the soil (SHANER et al., 2012). Therefore, mesotrione degradation is rapidly and essentially dependent on microbial action, with a DT50 from 3 to 26 d in all tested soils. The dissipation of mesotrione in soil is due to microbial metabolism-dependent and time sorption to soil (SHANER et al., 2012). DT50 of mesotrione in maize plants and soil were 1.37 and 4.31 d in Beijing, and 0.97 and 1.80 d in Shandong, China, respectively (CHEN et al., 2012). Therefore, mesotrione degradation process is dependent on the soil properties and types; and mesotrione also presents leaching potential (CHAABANE et al., 2008).

Juan et al. (2015) found that the highest concentration of mesotrione had an effect on soil microbial abundance and activity. Studies concerning the effects of soil management (amendment and herbicide application to agricultural soils) on microbial parameters are important for maintaining soil quality and its functioning. Likewise the study of the effect of amendment and herbicide application on soil microbial structure would be useful to complete our knowledge on this subject.

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[3] MENDES, K.F.; MARTINS, B.A.B.; REIS, F.C.; DIAS, A.C.R.; TORNISIELO, V.L. Methodologies to study the behavior of herbicides on plants and the soil using radioisotopes. **Planta Daninha,** Viçosa, 2017 (accepted manuscript).

Crouzet et al. (2010) studied its biodegradation and the soil microbial community's response to mesotrione applied at different doses in an unamended soil and two biodegradation products, 4-methylsulfonyl-2-nitrobenzoic acid (MNBA) and 2-amino-4-methylsulfonylbenzoic acid (AMBA) were detected.

The results lend support to the potential of fresh oiled and de-oiled two-phase olive mill waste amendments as an effective management practice to increase S-metolachlor persistence in soils (PEÑA et al., 2013). This increase does not necessarily ensure decreased leaching of the herbicide but it could also increase the risk of surface water contamination at higher application rate. However, its very low sorption by soil and relatively high water solubility (488 mg L^{-1}) means that it is frequently detected in ground and surface waters. Indeed, Konstantinou et al. (2006) found that this herbicide was one of the most important contaminants in terms of frequency of detection in waters of agricultural areas of several Mediterranean countries, including Greece, Italy, and Spain.

Because of its infiltration into the soil after heavy rain, terbuthylazine is one of herbicides most commonly found in Italian rivers and groundwater (SBRILLI et al., 2005). DT50 of terbuthylazine was measured in river and sea water samples of Murcia (southeast Spain), incubated in different laboratory conditions by Navarro et al. (2004), that found a DT50 with an interval between 76 and 331 d.

Herbicides applied pre-emergence in Europe, as terbuthylazine, it is often found in groundwater at concentrations higher than 0.1 μg L^{-1}, which is the maximum limit allowed by the European Community for drinking water (LANDRY et al., 2006). In intensive production areas of northern Italy, on the soil surface and water corn fields had the highest herbicide concentrations of triazines and its metabolites, and the terbuthylazine most often detected (FAVA et al., 2010). Hermosín et al. (2013) related the presence of various pesticides in surface and ground waters, including terbuthylazine, to rain events and runoff/leaching processes.

Results show by Otto et al. (2012) reported that without vegetative filter strip the herbicide load that reaches the surface water is about 5–6 g ha^{-1} $year^{-1}$ for S-metolachlor and terbuthylazine (i.e. 0.5–0.9% of the applied rate), confirming that runoff from flat fields as in the Po Valley (north-east Italy) can have a minor effect on the water quality, and that most of the risk is posed by a few, or even just one extreme rainfall event with a return period of about 25–27 years, causing runoff with a maximum concentration of 64–77 μg L^{-1}. The same authors found that mesotrione instead showed rapid soil disappearance and was observed at a concentration of 1.0–3.8 μg L^{-1} only after one extreme (artificial) rainfall. Vegetative filter strips of any type are generally effective and can reduce herbicide runoff by 80–88%. Their effectiveness is steady even under severe rainfall conditions, and this supports their implementation in an environmental regulatory scheme at a catchment or regional scale.

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3. SORPTION AND DESORPTION OF MESOTRIONE ALONE AND MIXED WITH S-METOLACHLOR + TERBUTHYLAZINE IN BRAZILIAN SOILS[4]

Resumo

A mistura de herbicidas é uma técnica amplamente utilizada no controle de plantas daninhas em diversas culturas, incluindo o milho. No entanto, as interações que podem potencialmente resultar da mistura de herbicidas são uma questão de preocupação constante e pesquisa. A capacidade do solo para reter herbicidas influencia diretamente o destino final destas moléculas no ambiente. O objetivo deste estudo foi avaliar a sorção e dessorção do mesotrione aplicada isolado e misturado com S-metolachlor + terbuthylazine em amostras de solos de sete áreas brasileiras cultivadas com milho. Sorção do mesotrione (isolado e misturado) foi avaliada com cinco concentrações de 0,125, 1,042 e 0,625 mg kg^{-1} correspondente à dose de campo do mesotrione, S-metolachlor e terbuthylazine, respectivamente. A sorção do mesotrione aplicado isolado e misturado apresentou valores de K_d (coeficiente de sorção) variando de 0,08 a 5,05 kg de L^{-1} e de 0,09 a 5,20 kg L^{-1}, respectivamente, com comportamento semelhante entre os solos. A sorção do mesotrione foi influenciada principalmente pelo teor de argila mineral (CM) e o pH do solo. O modo de aplicação (isolado ou misturado) não influenciou a sorção ou dessorção. A sorção do mesotrione foi relativamente baixa nos solos, que gera um potencial de lixiviação em áreas produtoras de milho.

Palavras-chave: mistura de herbicida, propriedades físico-químicas, retenção do solo, ácido fraco.

Abstract

The mixture of herbicides is a widely used technique in weed control in several crops, including maize. However, interactions that might potentially result from mixing herbicides are a matter of constant concern and research. Soil capacity to retain herbicides directly influences the final destination of these molecules in the environment. The aim of this study was to evaluate sorption and desorption of mesotrione applied alone and mixed with S-metolachlor + terbuthylazine in soil samples from seven Brazilian sites cultivated with maize. Mesotrione sorption (alone and mixed) was evaluated five concentrations with 0.125, 1.042 and 0.625 mg kg^{-1} corresponding to the field dose of mesotrione, S-metolachlor and terbuthylazine, respectively. Sorption of mesotrione applied alone and mixed had K_d (sorption coefficient) values ranging from 0.08 to 5.05 kg L^{-1} and from 0.09 to 5.20 kg L^{-1}, respectively, with similar behavior across soils. Mesotrione sorption was influenced primarily by the clay mineral (CM) content and the soil pH. The mode of application (alone or mixed) did not influence sorption or desorption. Mesotrione sorption was relatively low in soils, which creates the potential for leaching in maize producing areas.

Keywords: herbicide mixture, physicochemical properties, soil retention, weak acid.

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[4] MENDES, K.F.; REIS, M.R.; INOUE, M.H.; PIMPINATO, R.F.; TORNISIELO, V.L. Sorption and desorption of mesotrione alone and mixed with *S*-metolachlor + terbuthylazine in Brazilian soils. **Geoderma**, Amsterdam, v.280, p.22-28, 2016.

3.1. Introduction

The environmental behavior of herbicides is determined by interactions occurring in herbicide-soil interfaces. These interactions primarily determine the bioavailability of the herbicide, which influences the sorption-desorption, volatilization, transformation by biotic and abiotic agents, and the possibility of contamination of ground and surface waters by through leaching and runoff (PATAKIOUTAS; ALBANIS, 2002).

The sorption of herbicides in soils is one of the most important processes influencing the bioavailability of these molecules. The degree of herbicide sorption and desorption in the soil is influenced by soil properties, such as organic matter content, texture, mineralogy, ion exchange capacity and pH. Herbicide sorption and desorption in the soil is also influenced by properties of the herbicide molecule, such as water solubility, the octanol-water partition coefficient and the dissociation constant. Determination of herbicide sorption is essential to study the factors that regulate herbicide-soil interactions (KAH; BROWN, 2006; CHIRUKURI; ATMAKURU, 2015).

Mesotrione {2-(4-methylsulfonyl-2-nitrobenzoyl)-1,3-cyclohenanedione}, a triketone herbicide, is chemically derived from natural allelochemicals (leptospermone) produced by the plant *Callistemon citrinus*. Mesotrione operates in the biosynthesis of carotenoids by interfering with the activity of the enzyme HPPD (4-hydroxyphenylpyruvate-dioxygenase) in the chloroplast, causing the bleaching of sensitive weeds and subsequent necrosis and death of plant tissues in approximately 1 to 2 weeks (MITCHELL et al., 2001; BEAUDEGNIES et al., 2009).

The behavior of ionizable herbicides in the soil (weak acids and bases) is more complex compared to neutral herbicides because they depend on soil pH, organic matter content, the type of clays and the presence of hydroxide and oxide of Fe/Al (KAH; BROWN, 2006). Sorption of the weak acid mesotrione involves the soil minerals and organic constituents, including fulvic acids. However, the interactions between the herbicide and soil properties are weak and correlate negatively with soil pH, resulting in desorption of mesotrione (DYSON et al., 2002; ALEKSEEVA et al., 2014).

The commercial herbicide mixture Lumax® (mesotrione + S-metolachlor + terbuthylazine) is a recommended pre-emergent herbicide in maize. Pinna et al.

(2014) studied this herbicide mixture by examining each molecule individually. The increasing use of Lumax® and, in particular, mesotrione, provides pre- and post-emergence control of dicotyledonous weeds and some annual grasses in maize.

Little is known about herbicide mixture effects in the soil, especially in tropical regions like Brazil. Studies regarding the behavior of herbicides in the soil are usually carried out considering single molecules. However, there may be possible interactions of additivity, synergism and antagonism among herbicide molecules in a mixture, as discussed by Bonfleur et al. (2015). Therefore, studies are needed to evaluate the effect of mixtures on herbicide sorption under different climate and soil conditions.

The aim of this study was to evaluate sorption and desorption of mesotrione applied alone and mixed with S-metolachlor + terbuthylazine in seven Brazilian soils from areas cultivated with maize. Results from this study will aid the understanding of key parameters responsible for the behavior of mesotrione in agricultural areas of Brazil.

3.2. Materials and Methods

3.2.1. Fractionation and characterization of soil

The sorption and desorption experiments of ^{14}C-mesotrione herbicide applied alone and in mixture with S-metolachlor + terbuthylazine 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 in this study followed the guidelines of the Organization for Economic Co-operation and Development (OECD, 2000).

Samples from soils that had not been treated with the herbicides mesotrione, S-metolachlor and terbuthylazine for the past 2 years were collected from the surface layer (0-10 cm of depth) in seven different maize producing areas in Brazil. After drying, the samples were sieved through a 2.0 mm sieve and stored at ambient temperature. Soil samples were sterilized by gamma irradiation (30 kGy) to inhibit microbial activity for potential degradation during the sorption and desorption experiments (SHANER et al., 2012). The physicochemical properties of the samples and the classification of the soils are shown in Table 3.1.

Table 3.1 - Physicochemical properties of soils (0-10 cm of depth) cultivated with maize in brazil studied in this experiment.

Soil	Origin (city, state, geographic coordinates)	Soil classification - symbology[a]	K	Ca^{2+}	Mg^{2+}	H + Al	BS	CEC
			($mmol_c$ kg^{-1})					
BR1	Rio Paranaíba, MG (S 19° 12’ 29”; W 46° 07’ 57”)	Oxisol - Rhodic Hapludox (Latossolo Vermelho distroférrico - LVdf)	11	70	16	57	97	154
BR2	Barra do Bugres, MT (S 15° 07’ 25’’; W 57° 17’ 21’’)	Entisol - Typic Quartzipsamments (Neossolo Quartzarênico órtico - RQo)	1	11	3	29	15	44
BR3	Barra do Bugres, MT (S 15° 04’ 39’’; W 57° 10’ 51’’)	Entisol - Typic Quartzipsamments (Neossolo Quartzarênico órtico - RQo)	2	47	6	29	55	84
BR4	Tangará da Serra, MT (S 14° 39’ 01”; W 57° 25’ 54”)	Oxisol - Typic Hapludox (Latossolo Vermelho distrófico - LVd)	4	25	11	67	40	107
BR5	Tangará da Serra, MT (S 14° 39’ 55”; W 57° 28’ 05”)	Oxisol - Typic Hapludox (Latossolo Vermelho distrófico - LVd)	14	39	23	40	76	116
BR6	Piracicaba, SP (S 22° 42’ 34”; W 47° 37’ 18”)	Alfisol - Paleudult (Nitossolo Vermelho eutroférrico - NVef)	11	51	26	41	88	129
BR7	Piracicaba, SP (S 22° 42’ 52”; W 47° 37’ 10”)	Ultisol - Typic Hapludalf (Argissolo Vermelho-Amarelo distrófico - PVAd)	1	18	7	29	26	55

Soil	pH (H_2O)	P (mg kg^{-1})	V (%)	OC (g kg^{-1})	VFS	FS	MS	CS	VCS	TS	CM	S	Texture class
					(g kg^{-1})								
BR1	6.4	67	63	27.32	41	148	87	16	2	294	509	196	clay
BR2	7.7	9	34	0.58	130	489	262	31	20	932	50	18	sand
BR3	7.3	19	65	4.07	191	448	202	10	2	853	124	23	loamy sand
BR4	6.0	4	37	22.09	71	143	60	7	1	282	605	113	clay
BR5	6.7	55	66	12.21	86	302	196	29	4	617	324	59	sandy clay loam
BR6	6.4	18	68	18.02	100	254	86	19	7	466	376	158	sandy clay
BR7	6.9	15	47	5.23	210	478	109	13	6	816	151	33	sandy loam

[a] According to Soil Taxonomy and Brazilian Soil Science Society (EMBRAPA, 2013). [14] K = potassium; Ca = calcium; Mg = magnesium; H + Al: potential acidity; BS = base saturation; CEC = cation exchange capacity; pH = potential of hydrogen; P = phosphorus; V = base saturation levels; OC = organic carbon; VFS = very fine sand; FS = fine sand; MS = medium sand; CS = coarse sand; VCS = very coarse sand; TS = total sand; CM = clay mineral and S = silt.

Source: Soil Science Department - ESALQ/USP, Piracicaba, SP, Brazil.

3.2.2. Experimental design

The experiment was completely randomized under a factorial arrangement of seven soil types, five concentrations and two mesotrione application modes (mesotrione alone and mixed). Three replications were considered. Each experimental unit consisted of a 50 mL Teflon tube with a screw cap. Aliquots, each with 10 g of soil, were weighed in triplicate in the tubes and 10 mL of calcium chloride solution ($CaCl_2$) 0.01 mol L^{-1} was used, resulting in a soil-solution ratio of 1:1 (m/v).

3.2.3. Chemical products

The stock solutions (170 mL) were prepared using non-radiolabeled analytical standards of mesotrione (150 g ha^{-1}), S-metolachlor (1,250 g ha^{-1}) and terbuthylazine (750 g ha^{-1}) with purities of 99.9, 98.2 and 98.8%, respectively (Sigma Aldrich, Saint Louis, MO, USA). The analytical standard ^{14}C-mesotrione (Izotop, Budapest, Hungary) showed 98.4% radiochemical purity and 3.45 MBq mg^{-1} of specific activity. Radiolabeled and non-radiolabeled standards were carefully mixed in 0.01 mol L^{-1} $CaCl_2$. Working solutions had concentrations of 1.25, 2.50, 5.00, 7.50 and 10.00 mg L^{-1}, and contained, respectively, 0.28, 0.57, 1.13, 1.70 and 2.27 MBq L^{-1} of specific activity for mesotrione.

3.2.4. Mesotrione sorption–desorption experiments

In the sorption experiments, 120 µL aliquots of radiolabeled solutions were transferred in duplicate to separate vials containing 10 mL of the scintillation solution insta-gel plus, and the initial concentration of ^{14}C-mesotrione after 15 min was determined by liquid scintillation counting (LSC) with a Tri-Carb 2910 TR LSA counter (PerkinElmer).

In duplicate, 10 mL of the radiolabeled concentrations of all solutions were added to the Teflon tubes containing the soil samples. The tubes were agitated in a horizontal shaker tabletop in a dark room (20 ± 2 °C) for 24 h (time determined in preliminary experiments - data not published) to achieve the equilibrium concentration (DYSON et al., 2002; SHANER et al., 2012).

At the equilibration concentration, the tubes were centrifuged at 755 *g* for 15 min, and 1 mL aliquots of the supernatant from each tube were transferred in duplicate to scintillation vials containing 10 mL of the scintillation solution Insta-gel

Plus and analyzed by LSC to determine the concentration of the ^{14}C-mesotrione solution by counting the radioactivity. The herbicide sorbed amount was calculated using the difference between the initial concentration and the concentration in the supernatant after equilibration.

Desorption experiments were performed immediately after sorption under the same conditions. For that, $CaCl_2$ solution (10 mL, 0.01 mol L^{-1}) was added to the Teflon tubes containing the soil and the radiolabeled herbicide sorbed from the sorption experiment. The tubes were agitated in a horizontal shaker tabletop in a dark room (20 ± 2 °C) for 24 h to reach the equilibrium concentration. After re-equilibration, the tubes were centrifuged and 1 mL aliquots of the supernatant were pipetted in duplicate to scintillation vials containing 10 mL of the scintillation solution and analyzed by LSC. The desorbed amount was calculated as the difference between the radioactivity sorbed in the soil and in the remaining supernatant.

To evaluate the possible adherence of the radiolabeled material to the walls of the tubes, the experiments contained two controls: 1) tubes with a solution of 0.01 mol L^{-1} $CaCl_2$ and soil; and 2) tubes with the radiolabeled solution at all of the concentrations added to the tube without soil.

The Teflon tubes of soil were dried at 40 °C for 48 h. Then, the tubes were ground and stored in plastic containers. Subsamples of each soil were weighed in triplicate (0.2 g - dry basis) and oxidized in a biological oxidizer OX500 (R.J. Harvey Instrument Corporation) to determine the amount of residues in the soil that had not been extracted, as well as the amount of residues that could be extracted by solvents. Concentration of ^{14}C was determined in a LSC.

3.2.5. Mesotrione sorption-desorption model

Sorption coefficients K_f and $1/n$ were calculated from the slope and intercept of the Freundlich equation: $C_s = K_f \times C_e^{1/n}$; where C_s is the concentration (µmol kg^{-1}) of mesotrione sorbed onto the soil after equilibration; K_f is the equilibrium constant Freundlich (µmol $^{(1-1/n)}$ $L^{1/n}$ kg^{-1}); C_e is the mesotrione concentration (µmol L^{-1}) after equilibration; and $1/n$ is the degree of linearity of the isotherm. The sorption distribution coefficients (K_d, L kg^{-1}), where $K_d = C_s/C_e$, and the sorption coefficient normalized to the OC content of the soil (K_{oc}, L kg^{-1}), where $K_{oc} = (K_d/(\%OC)) \times 100$ were also calculated. The equilibrium constant K_{foc} sorption standard for the OC content of the soil was adjusted using the following formula: $K_{foc} = (K_f/(\%OC)) \times 100$. The desorption coefficients K_f and $1/n$ were determined in a similar manner to the sorption coefficients using a plot of the amount of remaining chemical sorbed at each desorption step versus the equilibrium concentration. The desorption K_d value was also calculated for comparison to the sorption K_d. The hysteresis coefficient (H) for the sorption-desorption isotherms was calculated according to the formula $H= (1/n_{desorption})/(1/n_{sorption})$, where $1/n_{sorption}$ and $1/n_{desorption}$ are the Freundlich slopes obtained for the sorption and desorption isotherms, respectively (BARRIUSO et al., 1994).

3.2.6. Statistical data processing

An analysis of variance (ANOVA) was used to detect differences in mesotrione sorption or desorption coefficients within each soil and application mode (alone or mixed). When significant, means were compared by Tukey test (*p*=0.05). The Pearson correlation coefficients were estimated between the sorption coefficients (K_d and K_{oc}) and soil properties (cation exchange capacity, CEC; potential of hydrogen, pH, organic carbon, OC and clay mineral content, CM). When significant, linear regressions were plotted using Sigma Plot® (Version 10.0 for Windows, Systat Software Inc., Point Richmond, CA).

3.3. Results and Discussion

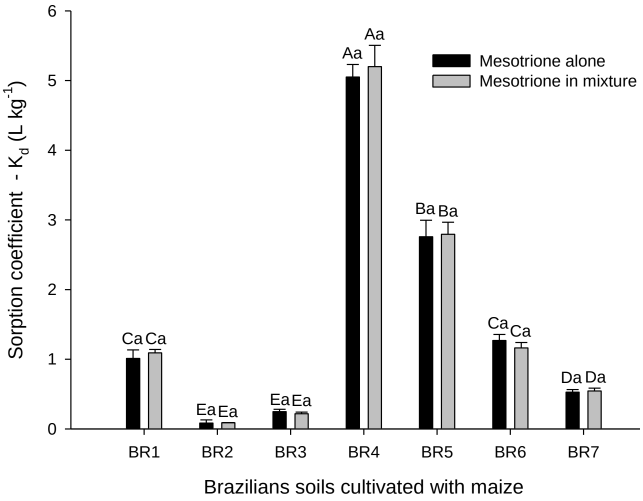
3.3.1. Sorption of mesotrione alone and mixed

The triple interaction between soil type, mesotrione concentration and application mode (alone or mixed) was not statistically significant, and only the interaction between soil type and application mode was significant (Figure 3.1). Mixing ^{14}C-mesotrione with S-metolachlor and terbuthylazine showed no effect on mesotrione retention in the soil, as there were no significant differences in K_d and K_{oc} across soils (Figures 3.1 and 3.2, respectively).

In herbicide sorption studies, K_d calculation usually takes into account herbicide-soil interactions, as a phenomenon that occurs evenly throughout the volume of soil (GOMES et al., 2002). High K_d values may indicate high herbicide retention by the soil (OLIVEIRA et al., 2004).

Sorption of mesotrione applied alone and mixed with S-metolachlor + terbuthylazine showed K_d values ranging from 0.08 to 5.05 L kg^{-1} and 0.09 to 5.20 L kg^{-1}, respectively. Whether applied alone or mixed, mesotrione retention in the soil was similar. The lower K_d values were found in an Entisol - Typic Quartzipsamments (BR2) and an Entisol - Typic Quartzipsamments (BR3) (Figure 3.1), which are sandy soils with a clay mineral (CM) content of less than 13% (Table 3.1). On the other hand, the higher K_d values were reported in an Oxisol - Typic Hapludox (BR4) with clay texture. There was no difference in K_d values between the Oxisol - Rhodic Hapludox (BR1) and the Alfisol - Paleudult (BR6), with clay and sandy clay textures, respectively. Soils BR1 and BR6 are from different regions, Rio Paranaíba/MG and Piracicaba/SP, respectively (Figure 3.1).

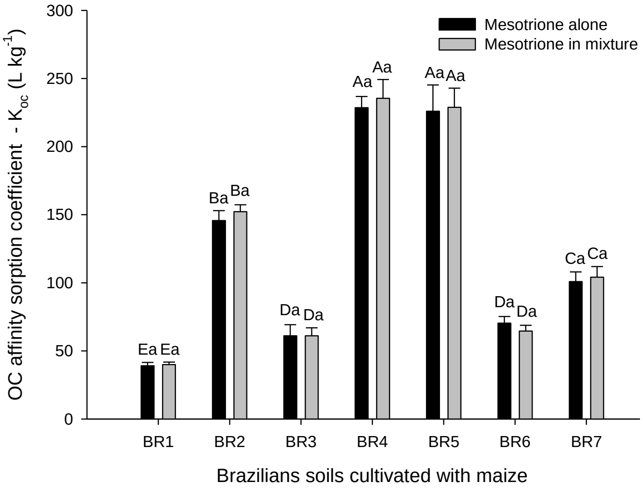
Figure 3.1 - Sorption coefficient - K_d (L kg^{-1}) of mesotrione applied alone and mixed with S-metolachlor + terbuthylazine in Brazilians soils cultivated with maize, represented by BR1 (Oxisol - Rhodic Hapludox), BR2 (Entisol - Typic Quartzipsamments), BR3 (Entisol - Typic Quartzipsamments), BR4 (Oxisol - Typic Hapludox), BR5 (Oxisol - Typic Hapludox), BR6 (Alfisol - Paleudult) and BR7 (Ultisol - Typic Hapludalf). The vertical bars associated with each column represent the standard deviation ($\pm$SD) of each mean value (*n* = 3). Means followed by the same capital letter in each soil and tiny in herbicide alone/mixtures do not differ by Tukey test ($p<0.05$). DMS (soil) = 0.18205, DMS (herbicide alone/mixture) = 0.06382 and CV (%) = 8.46.



In a study with mesotrione that covered several soil types and textures with different pH and OC levels, Dyson et al. (2002) found mesotrione K_d values ranging from 0.13 to 5.0 L kg^{-1}. Shaner et al. (2012) observed mesotrione K_d values ranging from 0.26 to 3.53 L kg^{-1} in four soils, similar to data presented in this work.

The K_{oc} values for mesotrione applied alone and mixed ranged from 39.05 to 228.61 L kg^{-1} and 39.94 to 235.43 L kg^{-1}, respectively, with higher values for the Oxisol - Typic Hapludox (BR4) and the Oxisol - Typic Hapludox (BR5). Mesotrione K_{oc} values were lower for the Oxisol - Rhodic Hapludox soil (BR1) (Figure 3.2). K_{oc} values of mesotrione were similar between the Entisol - Typic Quartzipsamments (BR3) and Alfisol - Paleudult (BR6) soils, and ranged between 61 and 70 L kg^{-1} (Figure 3.2). Dyson et al. (2002) found mesotrione K_{oc} values ranging from 15 to 390 L kg^{-1}.

Figure 3.2 - OC affinity sorption coefficient - K_{oc} (L kg^{-1}) of mesotrione applied alone and mixed with S-metolachlor + terbuthylazine in Brazilians soils cultivated with maize, represented by BR1 (Oxisol - Rhodic Hapludox), BR2 (Entisol - Typic Quartzipsamments), BR3 (Entisol - Typic Quartzipsamments), BR4 (Oxisol - Typic Hapludox), BR5 (Oxisol - Typic Hapludox), BR6 (Alfisol - Paleudult) and BR7 (Ultisol - Typic Hapludalf). The vertical bars associated with each column represent the standard deviation (±SD) of each mean value (*n* = 3). Means followed by the same capital letter in each soil and tiny in herbicide alone/mixtures do not differ by Tukey test ($p<0.05$). DMS (soil) = 12.20393, DMS (herbicide alone/mixture) = 4.27841 and CV (%) = 7.14.



K_{oc} values enable a comparison of sorption among different soils, and is generally used in mobility classification methods and simulation models of pesticide behavior in soils. This type of K_d standardization is important specially for herbicides that have their sorption directly influenced by soil organic matter (OLIVEIRA JUNIOR et al., 2001).

In Brazil, K_{oc} has been widely used to predict the sorption capacity of various herbicides in the soil (SILVA et al., 2007), and is used to optimize herbicide dose, given K_{oc} is an index related to soil organic matter. However, there is no consensus among researchers about standardizing K_d in relation to soil OC because the herbicide sorption to soil OM occurs unevenly, and depends on the mechanisms and the organic fraction involved in the sorption process. At the same time, K_d and K_{oc} are not always sufficient to accurately describe the sorption of a herbicide in a given concentration range.

The Freundlich equations adequately described the sorption of mesotrione applied alone and mixed with S-metolachlor + terbuthylazine in all soils ($R^2 \geq 0.92$). K_f values were not dependent on mesotrione concentration, but varied across soil types and did not differ between the two application modes considered. In addition, sorption values of $1/n_{sorption}$ were close to 1.0 (Table 3.2), indicating the constant partitioning of mesotrione between the adsorbent and the soil solution in the studied concentration ranges. In general, the K_f values of sorption were low, ranging from 0.10 to 0.12 (BR2) and 4.01 to 4.46 µmol $^{(1-1/n)}$ $L^{1/n}$ kg^{-1} (BR4) for mesotrione applied alone and mixed, respectively (Table 3.2), in accordance with the K_d values shown above (Figure 3.1). For the K_{foc} values in relation to the soil OC, the means ranged from 28.92 (BR1) to 235.87 µmol $^{(1-1/n)}$ $L^{1/n}$ kg^{-1} (BR5) for the mesotrione applied mixed (Table 3.2), similar to the K_{oc} values described in Figure 3.2.

Table 3.2 - Freundlich sorption parameters for mesotrione applied alone and mixed with S-metolachlor + terbuthylazine in Brazilians soils cultivated with maize.

Soil[a]	Mesotrione	$K_{f\ (sorption)}$	$K_{foc\ (sorption)}$	1/n $_{(sorption)}$	R^2
		(µmol $^{(1-1/n)}$ $L^{1/n}$ kg^{-1})			
BR1	alone	0.95 (0.88-1.03)[b]	34.77 (32.21-37.70)	0.96 ± 0.02[c]	0.94
	mixture	0.79 (0.60-1.03)	28.92 (21.96-37.70)	0.89 ± 0.04	0.98
BR2	alone	0.10 (0.08-0.12)	172.41 (137.93-206.90)	1.07 ± 0.05	0.96
	mixture	0.12 (0.11-0.13)	206.90 (189.65-224.14)	1.11 ± 0.01	0.99
BR3	alone	0.38 (0.25-0.47)	93.37 (61.42-115.47)	0.77 ± 0.09	0.93
	mixture	0.28 (0.27-0.30)	68.80 (66.34-73.71)	0.81 ± 0.06	0.98
BR4	alone	4.01 (3.19-5.06)	181.53 (144.40-229.06)	0.94 ± 0.09	0.98
	mixture	4.46 (4.14-4.85)	201.90 (187.41-219.56)	0.81 ± 0.05	0.99
BR5	alone	2.23 (2.03-2.39)	182.64 (166.26-195.74)	0.79 ± 0.07	0.96
	mixture	2.88 (2.40-3.13)	235.87 (196.56-256.34)	1.01 ± 0.04	0.94
BR6	alone	1.09 (0.91-1.39)	60.49 (50.50-77.14)	0.94 ± 0.05	0.92
	mixture	1.11 (0.78-1.26)	61.59 (43.28-69.92)	0.78 ± 0.08	0.98
BR7	alone	0.54 (0.39-0.63)	103.25 (74.57-120.45)	1.01 ± 0.02	0.92
	mixture	0.47 (0.43-0.58)	89.87 (82.21-110.89)	0.96 ± 0.05	0.94

[a] BR1, Oxisol - Rhodic Hapludox; BR2, Entisol - Typic Quartzipsamments; BR3, Entisol - Typic Quartzipsamments; BR4, Oxisol - Typic Hapludox; BR5, Oxisol - Typic Hapludox; BR6, Alfisol - Paleudult and BR7, Ultisol - Typic Hapludalf.

[b] Number in parentheses are confidence intervals of the mean (K_f and K_{foc}), $n = 3$.

[c] Mean 1/n value ± standard deviation of the mean.

Pinna et al. (2014) studied the active ingredients of the commercial herbicide Lumax®, composed by mesotrione + S-metolachlor + terbuthylazine, and found that the K_f values measured for mesotrione ranged from 0.34 to 0.44 µmol $^{(1-1/n)}$ L$^{1/n}$ kg^{-1}, with the application of mineral and organic fertilizers to the soil (OM 1.67 to 2.00% and 3% clay), indicating a weak affinity between the mesotrione and the soil surface, which was predominantly loaded negatively due to the pH range of the analyzed soils (5.58 to 6.48). The authors also stated that the values of the Freundlich exponent ($1/n_{sorption}$) for mesotrione in all absorbers were always $\leq$ 1, justifying a sorption isotherm similar to the L type (GILES et al., 1960). The curve of type L indicates a relatively greater affinity of mesotrione in low concentrations in the soil. With an increasing herbicide concentration, the intensity of the sorption decreases due to the low bioavailability of empty sorption sites for mesotrione. In general, in accordance with the K_f values observed by Pinna et al. (2014), the sorption of the three herbicides followed the mesotrione < S-metolachlor < terbuthylazine order. This trend is closely linked to the octanol-water partition coefficient (K_{ow}) of the components of Lumax®, where the hydrophobic interaction influences on the sorption of herbicides in soil.

3.3.2. Desorption of mesotrione applied alone and mixed

The desorption K_f values ranged from 3.96 (BR7) to 12.65 µmol $^{(1-1/n)}$ L$^{1/n}$ kg^{-1} (BR4) when mesotrione was applied mixed with S-metolachlor + terbuthylazine. The desorption K_f values were similar to the K_d values, which varied from 4.06 (BR7) to 13.77 L kg^{-1} (BR2) (Table 3.3). No difference was observed between mesotrione application modes ($p>0.05$).

The Freundlich equations adequately described the desorption of mesotrione applied alone and mixed with S-metolachlor + terbuthylazine for all soils ($R^2 \geq 0.90$) (Table 3.3). The desorption values of $1/n_{desorption}$ ranged between 0.28 and 0.73 for all soils, regardless the application mode.

The desorption was hysteretic for both mesotrione application modes in all soils ($1/n_{desorption} < 1/n_{sorption}$) (Tables 3.2 and 3.3). According to Koskinen et al. (2006), this fact means that it is difficult to desorb the previously sorbed herbicide, and the desorption cannot be predicted from the sorption isotherms.

Hysteresis coefficient (H) is a measure of hysteresis during herbicide desorption (PINNA et al., 2014). H values below 1 indicate that the desorption percentage is less than that of the sorption and that hysteresis occurs. The H values for mesotrione applied alone ranged from 0.32 to 0.75 and from 0.35 to 0.77 for mesotrione applied mixed (Table 3.3). There was no correlation between H and K_f across soils. However, correlations were observed between H and the physicochemical properties of the soils. According to the data, it appears that mesotrione was sorbed and did not return to the soil solution quickly, as observed in other studies (OLIVEIRA JUNIOR et al., 2011; WAUCHOPE et al., 2002). Similar to our study, mesotrione H values ranged from 0.03 to 0.38 (PINNA et al., 2014).

Table 3.3 - Freundlich desorption parameters, desorption coefficient (K_d) and hysteresis coefficient (H) for mesotrione applied alone and mixed with S-metolachlor + terbuthylazine in Brazilians soils cultivated with maize.

Soil[a]	Mesotrione	$K_{f\ (desorption)}$ (µmol $^{(1-1/n)}$ L$^{1/n}$ kg^{-1})	$K_{d\ (desorption)}$ (L kg^{-1})	1/n (desorption)	R^2	H
BR1	alone	6.68 (6.07-7.25)[b]	7.57 ± 0.37[c]	0.72 ± 0.04[c]	0.93	0.75
	mixture	8.45 (7.45-8.65)	8.72 ± 0.25	0.69 ± 0.08	0.95	0.77
BR2	alone	11.29 (10.77-12.03)	13.01 ± 1.53	0.56 ± 0.05	0.90	0.52
	mixture	12.48 (11.39-12.91)	13.77 ± 1.24	0.62 ± 0.08	0.95	0.56
BR3	alone	8.11 (7.47-9.16)	8.83 ± 0.93	0.37 ± 0.10	0.93	0.48
	mixture	7.47 (6.59-8.44)	8.38 ± 0.56	0.48 ± 0.07	0.95	0.59
BR4	alone	12.45 (10.04-13.12)	13.33 ± 0.35	0.31 ± 0.11	0.99	0.32
	mixture	12.65 (11.87-12.74)	13.04 ± 0.30	0.28 ± 0.08	0.93	0.35
BR5	alone	11.78 (10.25-12.31)	12.94 ± 0.80	0.49 ± 0.04	0.98	0.62
	mixture	10.11 (9.96-11.41)	11.46 ± 0.61	0.73 ± 0.03	0.98	0.72
BR6	alone	5.03 (4.55-6.14)	6.47 ± 0.18	0.48 ± 0.08	0.98	0.51
	mixture	5.11 (4.96-5.41)	6.66 ± 0.11	0.33 ± 0.02	0.98	0.42
BR7	alone	4.08 (3.31-4.78)	4.53 ± 0.12	0.73 ± 0.07	0.98	0.73
	mixture	3.96 (3.11-4.41)	4.06 ± 0.03	0.67 ± 0.09	0.97	0.69

[a] BR1, Oxisol - Rhodic Hapludox; BR2, Entisol - Typic Quartzipsamments; BR3, Entisol - Typic Quartzipsamments; BR4, Oxisol - Typic Hapludox; BR5, Oxisol - Typic Hapludox; BR6, Alfisol - Paleudult and BR7, Ultisol - Typic Hapludalf.

[b] Number in parentheses are confidence intervals of the mean (K_f and K_{foc}), *n* = 3.

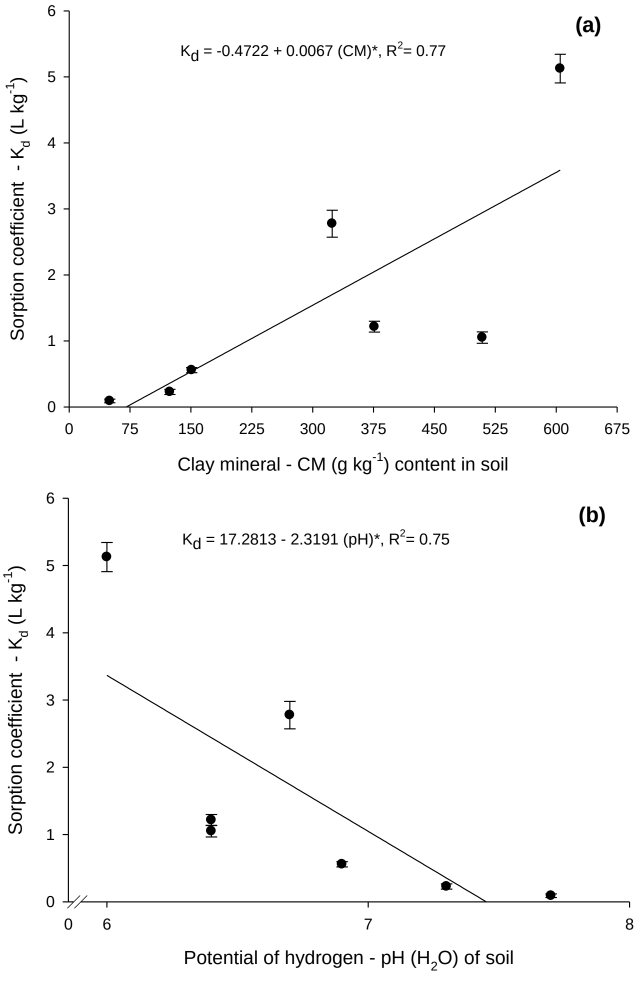
[c] Mean 1/n value ± standard deviation of the mean.

The K_d values for desorption were greater than those for sorption for all soils and mesotrione application modes (Table 3.3 and Figure 3.1, respectively). Based on the the sorption coefficients observed, mesotrione is very mobile in the soil. Marín-Benito et al. (2014), estimated K_d values for mesotrione sorption (0.03 to 0.48 L kg^{-1}).

3.3.3. Correlation between sorption coefficients and physicochemical properties of soils

K_d values for mesotrione applied alone or mixed with S-metolachlor + terbuthylazine were correlated with CM content and soil pH (Figure 3.3). Simple linear regression equations of K_d values as a function of CM content or soil pH were generated (Figures 3.3a and 3.3b, respectively). Linear equations that estimate K_d values considering the physicochemical properties of the soil may be helpful in the calculation of optimal doses for herbicide applications and should improve the predictability of the sorption of herbicides in soils with similar properties (MENDES et al., 2014; WEBER et al., 2000).

Figure 3.3 - Correlation between the sorption coefficient – K_d (L kg^{-1}) mean of mesotrione applied alone and mixed with S-metolachlor + terbuthylazine, and the clay mineral – CM (g kg^{-1}) content (a) and the potential of hydrogen – pH (H_2O) (b) of Brazilians soils cultivated with maize. The vertical bars associated with each symbol represent the standard deviation (+SD) of each mean value (*n* = 3). **p*<0.05 by the F test.



The absence of significant correlations between K_d values and the OC content in this experiment cannot be attributed to the result of a narrow-band OC soil (0.58 to 27.32 g kg^{-1}). The absence of significant correlations between K_d values and the OC content might be because mesotrione sorption is mostly related to the soil pH and, to a lesser extent, to the OC content, as described by Dyson et al. (2002).

Soil texture remains the reference parameter for the recommended dose of herbicides applied pre-emergence, as is the case of mesotrione applied alone or mixed with S-metolachlor + terbuthylazine or pre-plant incorporated (SILVA et al., 2007). However, the occurrence of several clay types and clay minerals in the formation of compounds in the soil represent different sorption possibilities of herbicides to such compounds.

Significant correlations between K_{oc} and the physicochemical properties of the soils were not observed across mesotrione application modes on mesotrione sorption in the soil. However, significant correlations between K_d values and the physicochemical properties of the soils were observed, making K_d the index of use when considering mesotrione sorption and physicochemical properties of the soils.

Positive correlations between K_d and CM content were similar between the application modes, with no significant difference (p>0.05) (Figure 3.3a). This fact may indicate that the behavior of mesotrione in the soil is not affected by the application mode.

The increase in CM content in the soil caused an overall increase in mesotrione sorption due to greater K_d values, where the addition of 100 g kg^{-1} CM to the soil (1%) corresponds to an increase in K_d values of approximately 0.20 L kg^{-1} (Figure 3.3a).

Work conducted by Alekseeva et al. (2014) reported that mesotrione sorption processes in the soil are complex, involving mineral and organic constituents, including fulvic acids, but the interactions between herbicide and soil components are weak, resulting in the complete reversibility mesotrione sorption, a process known as desorption.

Negative correlations between K_d and soil pH were similar whether mesotrione was applied alone or mixed with S-metolachlor + terbuthylazine, with no significant differences (p>0.05) (Figure 3.3b), as reported for the correlation with the CM content of the soil.

Greater soil pH values provide less mesotrione sorption whether applied alone or mixed. Consequently, mesotrione remains available in the soil solution, due to lower K_d values, where an increase of 1.0 in the soil pH, represents a reduction in K_d values of approximately 14.96 L kg^{-1} (Figure 3.3b). This can be explained because mesotrione is a weak acid whose molecular forms present the ability to donate protons and produce negatively charged ions. Soil pH is inversely correlated with the retention of many weak acid herbicides (WEBER et al., 2000). Similar results were obtained by Dyson et al. (2002) and Shaner et al. (2012), who found mesotrione sorption was negatively correlated with soil pH and positively correlated with soil OC content. With an increase in the soil pH, K_d values were lower, and the herbicide dissociated, passing to a molecular form from an anion form.

The pH is a very important measure that may interfere with the herbicide sorption processes, especially with those that have great ability to ionize (weak bases or weak acids). The influence of the soil pH on the herbicide retention process is closely related to the electrolytic dissociation capacity, that is, the pKa of the compounds (SILVA et al., 2007).

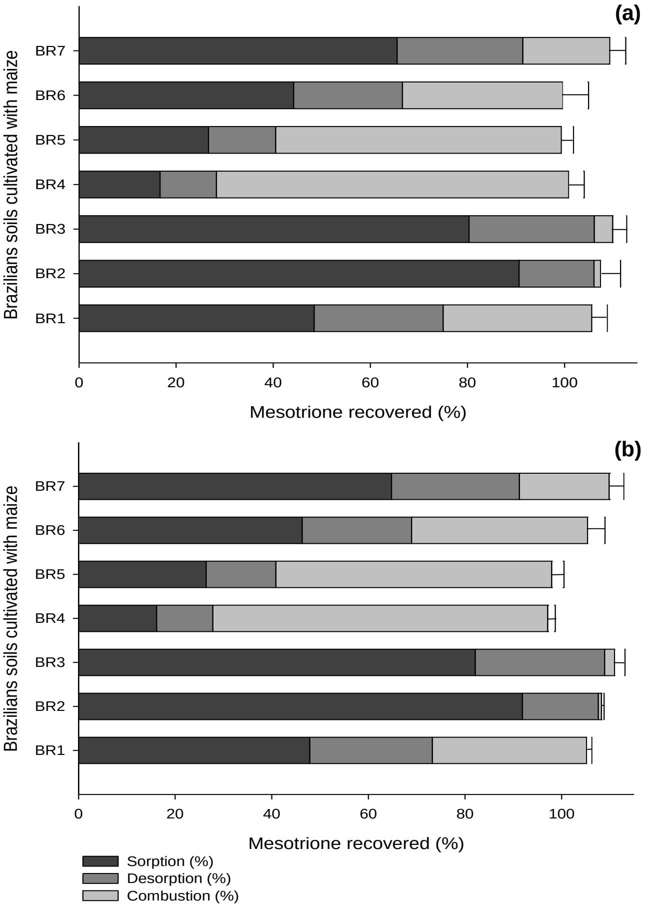
The pH levels above of pKa of the weak acid herbicides, including mesotrione (pKa = 3.12), which is substantially dissociated and not sorbed in this study soil, with a pH range from 6.0 to 7.7. Therefore, based on the mesotrione pKa, we should expect low sorption for all soils, as discussed in the section discussing K_d and K_{oc} values.

Oliveira Junior et al. (2001) reported that the weak acid herbicides (dicamba, imazethapyr, metsulfuron, nicosulfuron and sulfometuron), similar to mesotrione, showed less sorption compared to weak base herbicides (atrazine, simazine and hexazinone) and non-ionic herbicides (alachlor). In this case, acid soils generally have a greater mesotrione sorption capacity, and operations as liming in maize can significantly affect the behavior of this herbicide, especially its potential leaching, which can contaminate surface water and, subsequently, groundwater.

3.3.4. Mass balance of mesotrione applied alone and mixed

Mesotrione recovery was expressed by the total applied herbicide, represented by the sum of the percentage of sorbed, desorbed and combusted in the soil (Figures 3.4a and 3.4b). The quality control currently accepted admits an acceptable recovery range between 90 and 110% for the experiments using radiolabeled substances (OECD, 2000). Therefore, the recovery of mesotrione applied alone in this experiment is within acceptable values because it ranged between 99 (BR5) to 109% (BR3) (Figure 3.4a). The recovery of mesotrione applied mixed ranged between 97 (BR4) to 109% (BR3) (Figure 3.4b).

Figure 3.4 - Mass balance by the total applied mesotrione alone (a) and mixed with S-metolachlor + terbuthylazine (b) in Brazilians soils cultivated with maize, represented by BR1 (Oxisol - Rhodic Hapludox), BR2 (Entisol - Typic Quartzipsamments), BR3 (Entisol - Typic Quartzipsamments), BR4 (Oxisol - Typic Hapludox), BR5 (Oxisol - Typic Hapludox), BR6 (Alfisol - Paleudult) and BR7 (Ultisol - Typic Hapludalf). The vertical bars associated with each column represent the standard deviation (±SD) of each mean value (*n* = 3) of total mesotrione recovered.



When applied alone, mesotrione sorption and desorption percentages ranged from 16 (BR4) to 90% (BR2) and from 11 (BR4) to 26% (BR1), respectively (Figure 3.4a). In Belgium, using a sandy soil (23% clay, pH 6.6 and 2% OC), Chaabane et al. (2008) reported 30% desorption of mesotrione, suggesting that the sorption process was not fully reversible and resulted in the presence of a hysteresis phenomenon.

When applied mixed with S-metolachlor + terbuthylazine, mesotrione sorption and desorption percentages ranged from 16 (BR4) to 92% (BR2) and from 12 (BR4) to 27% (BR3), respectively (Figure 3.4b). Values for the combustion of soil samples ranged between 1 (BR2) and 72% (BR4) and 0.7 (BR2) to 69% (BR4) for mesotrione applied alone (Figure 3.4a) and mixed (Figure 3.4b), respectively, which may indicate the presence of bound residues (non-extractable).

It is well known that herbicides applied to the field are often easily bound to organic and mineral constituents of soil through physical and/or chemical interactions such as van der Walls forces, ligand exchange, charge-transfer complexes, hydrophobic partitioning, covalent bonding and sequestration, forming the so-called bound residue (GEVAO et al., 2000; BARRACLOUGH et al., 2005). Bound residues are compounds not amenable to extraction by methods that do not significantly alter the nature of the molecule and the matrix (FÜHR, 1987). Soil bound residues can be released again into the soil solution (remobilization) and may become available for uptake by plants and biotic communities, subsequently resulting in adverse effects such as phytotoxic effects on non-target plants (HAN et al., 2009).

In addition to the soil properties evaluated and correlated with K_d in this study, other herbicide sorption work in tropical soils correlated K_d values with climatic factors such as temperature and soil moisture, and edaphic factors, including humified part of OM, characteristics of clay minerals, iron and aluminum oxide (MENDES et al., 2014). It is important to note that iron and aluminum oxides also influence the sorption of various herbicides, particularly those with proton donating ability with negative charge (weak acid herbicides), as is observed for mesotrione.

3.4. Conclusions

Mesotrione, applied both alone and in a mixture with S-metolachlor + terbuthylazine, had no influence on its sorption or desorption. Both application modes exhibited similar retentions in the soil. Concurrently, mesotrione sorption in the soil is relatively low, indicating leaching potential, which can enter the groundwater in maize production fields.

Among the physicochemical properties of soils cultivated with maize, the pH and the CM content presented linear correlations with mesotrione sorption, allowing an understanding of key parameters responsible for the behavior of this herbicide in arable areas of Brazil. Our results should aid the predictability of mesotrione sorption in soils with similar properties.

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4. LEACHING MESOTRIONE ALONE AND IN A MIXTURE WITH S-METOLACHLOR AND TERBUTHYLAZINE IN TROPICAL SOILS[5]

Resumo

A lixiviação do herbicida é influenciada pelas propriedades físicas e químicas do solo, bem como as condições climáticas. No entanto, pouco se sabe sobre o comportamento de mistura de herbicidas no solo, especialmente em regiões tropicais como o Brasil. O objetivo deste estudo foi avaliar a lixiviação do mesotrione isolado e em mistura com S-metolachlor + terbuthylazine em sete solos tropicais e sua correlação com as propriedades físicas e químicas do solo. Estes solos representou uma grande variedade de propriedades com texturas diferentes, capacidade de troca catiônica (CTC) [44 a 154 $mmol_c\ kg^{-1}$], pH [6.0 a 7.7], teor de carbono orgânico (CO) [0,58 a 27,32 g kg^{-1}] e teor de argila mineral (CM) [50 a 605 g kg^{-1}] que são típicos de solos tropicais. Resíduos de mesotrione foram observados em todas as profundidades do solo (0-30 cm) e em todos os solos avaliados. Neste experimento, a forma de aplicação [mesotrione isolado ou em mistura] não influencia a lixiviação desse herbicida. A lixiviação de mesotrione é relativamente elevada nos solos tropicais e correlaciona-se com o pH (R^2 = -0,84) e teor de CM (R^2 = 0,75) e pode representar um potencial risco de contaminação das águas subterrâneas. Assim, as recomendações para a aplicação mesotrione, sem o conhecimento prévio das propriedades físicas e químicas do solo podem resultar em um ineficiente controle de plantas daninhas e de alto potencial de lixiviação.

Palavras-chave: ácido fraco, comportamento do solo, movimento descendente, propriedades físicas e químicas.

Abstract

Herbicide leaching is influenced by the soil physical and chemical properties as well as the climatic conditions. However, little is known about herbicide mixture behavior in the soil, especially in tropical regions like Brazil. The objective of this study was to evaluate the leaching of mesotrione alone and in a mixture with S-metolachlor plus terbuthylazine in seven tropical soils and their correlation with the soil physical and chemical properties. These soils represented a wide range of properties with varying textures, cation exchange capacity (CEC) [44 to 154 $mmol_c\ kg^{-1}$], pH [6.0 to 7.7], organic carbon (OC) content [0.58 to 27.32 g kg^{-1}] and clay mineral (CM) contents [50 to 605 g kg^{-1}] which are typical of tropical soils. Mesotrione residues were observed across all soil depths (0-30 cm) in all evaluated soils. In this experiment, the application form [mesotrione alone or in mixture] does not influence the leaching of this herbicide. Leaching of mesotrione is relatively high in the tropical soils and correlates with the pH (R^2 = -0.84) and CM content (R^2 = 0.75) and may pose a potential groundwater contamination risk. Thus, recommendations for mesotrione application, without the prior knowledge of the soil physical and chemical properties can result in an inefficient weed control and high leaching potentials.

Keywords: weak acid, soil behavior, downward movement, physical and chemical properties.

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[5] MENDES, K.F.; REIS, M.R.; SPOKAS, K.A.; TORNISIELO, V.L. Leaching mesotrione alone and in a mixture with S-metolachlor plus terbuthylazine in tropical soils. **Environmental Monitoring and Assessment**, Dordrecht, 2016 (submitted manuscript).

4.1. Introduction

Regardless of the herbicide application mode, pre- or post-emergence, they will eventually reach the soil. Thus, it becomes necessary to identify and understand herbicide interactions as a function of the soil physical and chemical properties. Herbicide movement in the soil system occurs in all directions and is dependent on the direction and rate of water infiltration and flow. Leaching is the name given to the downward movement of the herbicide to groundwater (PAULA et al., 2016; TONIÊTO et al., 2016).

Mesotrione, belonging to the family of triketones and pigment inhibitors, was one of the first agricultural herbicides to replace atrazine, and thereby quickly became the most widely used by farmers for weed control (NURSE et al., 2010). In addition, mesotrione is effective at controlling some weeds resistant to other herbicides (e.g. ALS - acetolactate synthase enzyme inhibitors and triazines). Mesotrione is generally applied in mixture with other herbicides such as S-metolachlor and terbuthylazine (PINNA et al., 2014; MILAN et al., 2015). However, mixtures of herbicides can alter sorption as well as microbial degradation rates which lead to alterations in the quantity of chemical leached (ROUCHAUD et al., 2001). This factor aids in explaining the observations of mesotrione in surface water and groundwater (ALFERNESS; WIEBE, 2002; MASTICHIADIS et al., 2003; FREITAS et al., 2004; BARCHANSKA et al., 2012).

The environmental behavior of mesotrione is positively correlated with the organic carbon (OC) contents and inversely correlated with the pH of the soil. This behavior is attributed to it being a weak acid [pKa = 3.1], i.e., dissociate into anion with increasing pH, these two parameters become mesotrione more or less available for transport and dissipation in soil (DYSON et al., 2002; SHANER et al., 2012; ALEKSEEVA et al., 2014).

The leaching of the commercial product Lumax®, comprising the mesotrione, S-metolachlor and terbuthylazine has been evaluated in soil from Italy (PINNA et al., 2014; MILAN et al., 2015). However, the potential interaction between herbicides when compared with application of each herbicide alone were not fully investigated. Many interactions are possible, including those related to changes in the amount and species diversity of the microbial population from different soils, effects on the specific enzymatic reactions, or those related to physical and chemical effects, such as competition for sorption sites, interfering directly in the leaching (SINGH et al., 2002; SWARCEWICZ; GREGORCZYK, 2012).

Given the above, the objective of this study was to evaluate the leaching of mesotrione alone and in mixture with S-metolachlor plus terbuthylazine in seven tropical soils and their correlation with the physical and chemical properties of soils.

4.2. Materials and methods

4.2.1. Fractionation and characterisation of the soil

The leaching experiments with ^{14}C-mesotrione applied alone and in mixture with S-metolachlor and terbuthylazine were performed in the Ecotoxicology Laboratory of the Center of Nuclear Energy in Agriculture (CENA), University of São Paulo, Piracicaba, Brazil. The methodology was established according to the guidelines of the Organization for Economic Co-operation and Development – 312, Leaching in Soil Columns (OECD, 2004). Tropical soil samples were collected from the surface layer (0–10 cm depth), after removing the vegetated layer, from areas cultivated with corn in Brazil. After drying, samples were sieved through a 2.0 mm mesh and stored at room temperature until use (~ 1 month).

4.2.2. Experimental design

We used a completely randomised design with three replications, which was then sub-divided into 2 × 7 × 6 sub-plots, where the factors were: two forms of mesotrione application [(a) alone and (b) mixture], seven types of tropical soils cultivated with corn and six soil depths.

4.2.3. Preparation of glass columns packed with soil

Three glass columns 50 cm in height and 5 cm in diameter were used for each sample of soil (three replications). The lower portion of the columns were filled with quartz wool, followed by a washed quartz sand layer before being dried in an oven at 100°C. Thereafter the soil samples were packed to a height of 30 cm, weighed to check the reproducibility of the packaging process of columns and the dose of mesotrione, with soil samples of BR1 (620.95 g), BR2 (1,003.73 g), BR3 (869.85 g), BR4 (670.95 g), BR5 (675.85 g), BR6 (791.00 g), and BR7 (921.74 g).

The soil columns were placed inside a 2.0 L beaker and were slowly wetted with an upward flow of $CaCl_2$ 0.01 mol L^{-1} solution. The columns were flooded for about 30 min. Subsequently, the columns were removed from the beaker and installed on a support and left for 2 h to drain the $CaCl_2$ solution.

4.2.4. Chemicals and application

Non-radiolabelled solutions were prepared using analytical standards of mesotrione, S-metolachlor and terbuthylazine of 99.9, 98.2 and 98.8% purity, respectively [Sigma Aldrich; Saint Louis, MO, USA]. The ^{14}C-mesotrione had a 98.4% radiochemical purity and specific activity equal to 3.45 MBq mg^{-1} [Izotop; Budapest, Hungary]. The solutions were prepared containing mesotrione (non-radiolabelled and radiolabeled) with 0.01 mol L^{-1} $CaCl^2$, applied alone and in mixture. Analytical standards were added (non-radiolabelled) at the maximum recommended dose of the active ingredient for corn: mesotrione (150 g ha^{-1}), S-metolachlor (1,250 g ha^{-1}) and terbuthylazine (750 g ha^{-1}). An aliquot of 200 μL of the herbicide solution was applied directly to the soil at the top of each column.

4.2.5. Leaching experiments of mesotrione alone and in mixture

After application of the herbicides a flow of 0.01 mol L^{-1} $CaCl_2$ solution at rate of approximately 8 mL h^{-1} was added for 48 h, resulting in a rain simulation of approximately 200 mm water depth.

At 12, 18, 24, 30, 36, 42 and 48 h after the administration three aliquots of 10 mL of the leaching were collected and added to 10 mL of solution calls gel for measurement for 15 min in the liquid spectrometer scintillation (LSS) with Tri-Carb 2910 TR LSA counter (PerkinElmer).

After 48 h of the herbicide application, the soil samples were removed from the columns by injecting air into the top of the column to force out the soil, which was subsequently cut into six equally sized sections (0–5, 5–10, 10–15, 15–20, 20–25 and 25–30 cm). The soil samples were air-dried, weighed, macerated and homogenised. Three sub-samples (0.2 g) of each dried layer of soil were biologically oxidised in an OX500 oxidizer (R. J. Harvey Instrument Corporation) for determination of total radioactivity.

The results are expressed as a % of the radioactivity found in the leachate and in each segment of the column, relative to the radioactivity initially applied. The recovery of the experiments was evaluated by the sum of ^{14}C-mesotrione percentages found in each soil segment and leachate. To verify the repeatability and analytical sensitivity of the method, samples of oxidised soil and leaching were performed in triplicate.

4.2.6. Statistical analysis

Data were subjected to analysis of variance (ANOVA). When significant, the means were compared by Tukey test ($p < 0.05$). Pearson correlation coefficients were estimated between leaching and soil properties (CEC, pH, OC and CM). When significant, linear regression between the leaching and soil properties were estimated and tested and plotted using the Sigma Plot program (version 10.0 for Windows, Systat Software Inc., Point Richmond, CA).

For data related to the leachate in the $CaCl_2$ solution, regression equations were adjusted ($p < 0.01$) by the F test, using the non-linear model with three parameter Gaussian equation: $\hat{y} = a \exp(-0{,}5((x-x_0)/b)^\wedge 2)$, in which a corresponds to the maximum percentage of leached mesotrione, x_0 to the time after application of the maximum percentage of leached mesotrione and b refers to the opening of the peak of the curve.

4.3. Results and Discussion

4.3.1. Soil characterisation

The physical and chemical properties of the samples and classification of soils are shown in Table 4.1.

Table 4.1 - Physicochemical properties of soils (0-10 cm of depth) cultivated with maize in brazil studied in this experiment.

Soil	Origin (city, state, geographic coordinates)	Soil classification - symbology[a]	K	Ca^{2+}	Mg^{2+}	H + Al	BS	CEC
			(mmol$_c$ kg^{-1})					
BR1	Rio Paranaíba, MG (S 19° 12’ 29”; W 46° 07’ 57”)	Oxisol - Rhodic Hapludox (Latossolo Vermelho distroférrico - LVdf)	11	70	16	57	97	154
BR2	Barra do Bugres, MT (S 15° 07’ 25’’; W 57° 17’ 21’’)	Entisol - Typic Quartzipsamments (Neossolo Quartzarênico órtico - RQo)	1	11	3	29	15	44
BR3	Barra do Bugres, MT (S 15° 04’ 39’’; W 57° 10’ 51’’)	Entisol - Typic Quartzipsamments (Neossolo Quartzarênico órtico - RQo)	2	47	6	29	55	84
BR4	Tangará da Serra, MT (S 14° 39’ 01”; W 57° 25’ 54”)	Oxisol - Typic Hapludox (Latossolo Vermelho distrófico - LVd)	4	25	11	67	40	107
BR5	Tangará da Serra, MT (S 14° 39’ 55”; W 57° 28’ 05”)	Oxisol - Typic Hapludox (Latossolo Vermelho distrófico - LVd)	14	39	23	40	76	116
BR6	Piracicaba, SP (S 22° 42’ 34”; W 47° 37’ 18”)	Alfisol - Paleudult (Nitossolo Vermelho eutroférrico - NVef)	11	51	26	41	88	129
BR7	Piracicaba, SP (S 22° 42’ 52”; W 47° 37’ 10”)	Ultisol - Typic Hapludalf (Argissolo Vermelho-Amarelo distrófico - PVAd)	1	18	7	29	26	55

Soil	pH (H_2O)	P (mg kg^{-1})	V (%)	OC (g kg^{-1})	VFS	FS	MS	CS	VCS	TS	CM	S	Texture class
					(g kg^{-1})								
BR1	6.4	67	63	27.32	41	148	87	16	2	294	509	196	clay
BR2	7.7	9	34	0.58	130	489	262	31	20	932	50	18	sand
BR3	7.3	19	65	4.07	191	448	202	10	2	853	124	23	loamy sand
BR4	6.0	4	37	22.09	71	143	60	7	1	282	605	113	clay
BR5	6.7	55	66	12.21	86	302	196	29	4	617	324	59	sandy clay loam
BR6	6.4	18	68	18.02	100	254	86	19	7	466	376	158	sandy clay
BR7	6.9	15	47	5.23	210	478	109	13	6	816	151	33	sandy loam

[a] According to Soil Taxonomy and Brazilian Soil Science Society (EMBRAPA, 2013). [14] K = potassium; Ca = calcium; Mg = magnesium; H + Al: potential acidity; BS = base saturation; CEC = cation exchange capacity; pH = potential of hydrogen; P = phosphorus; V = base saturation levels; OC = organic carbon; VFS = very fine sand; FS = fine sand; MS = medium sand; CS = coarse sand; VCS = very coarse sand; TS = total sand; CM = clay mineral and S = silt.

Source: Soil Science Department - ESALQ/USP, Piracicaba, SP, Brazil.

4.3.2. Leaching mesotrione alone and in mixture

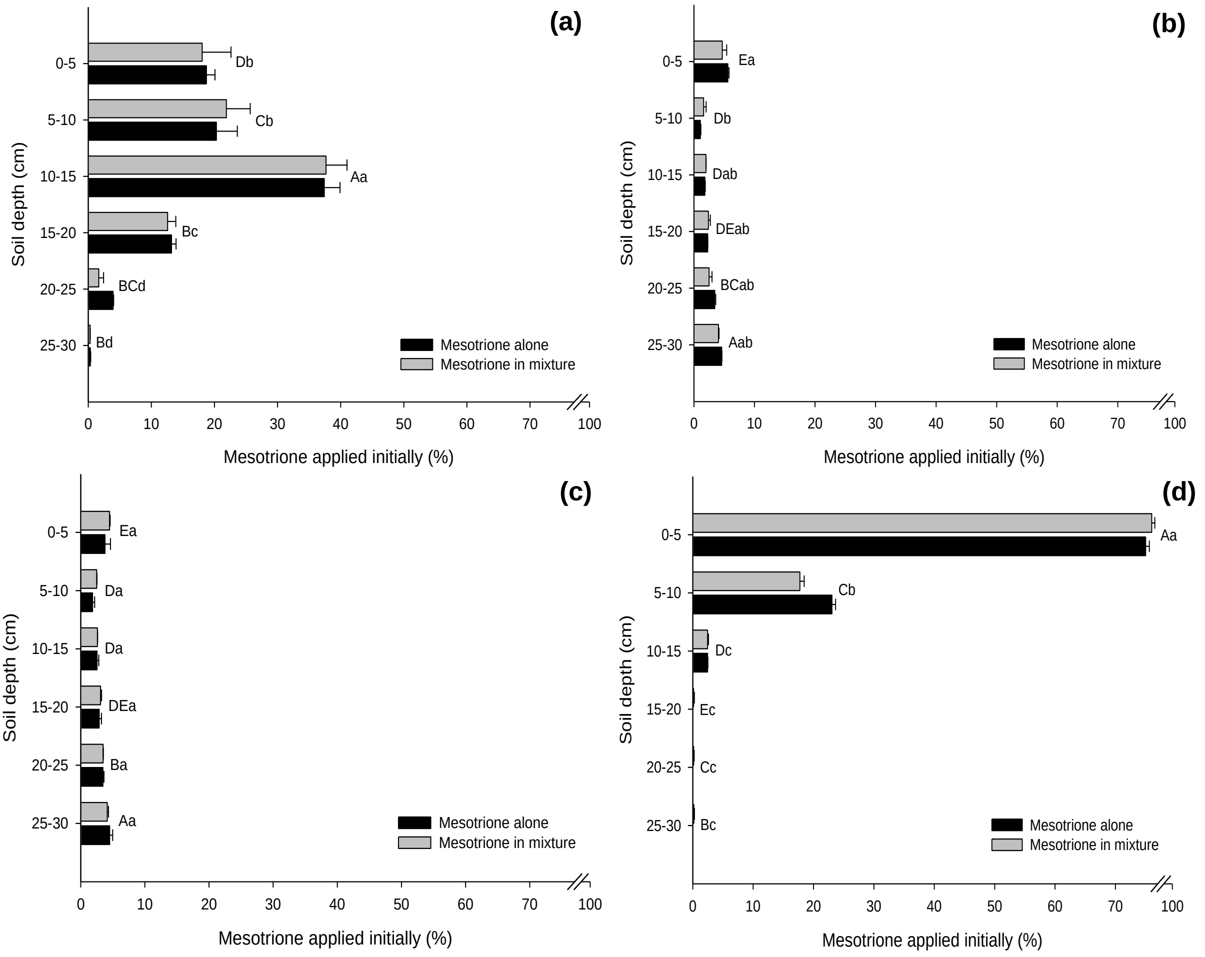
The triple interaction between the two preparations of mesotrione (alone and in mixture), the seven types of tropical soil and the six soil depths was not significant; indeed, significance was only observed for the double interaction between soil types and depths (Figure 4.1). The mesotrione preparation in mixture with S-metolachlor and terbuthylazine did not interfere with the leaching when compared with the application of mesotrione alone. This confirmed the results reported by Mendes et al. (2016), whereby the sorption coefficients with the same types of soils, showed no differences in mesotrione retention, regardless of application form.

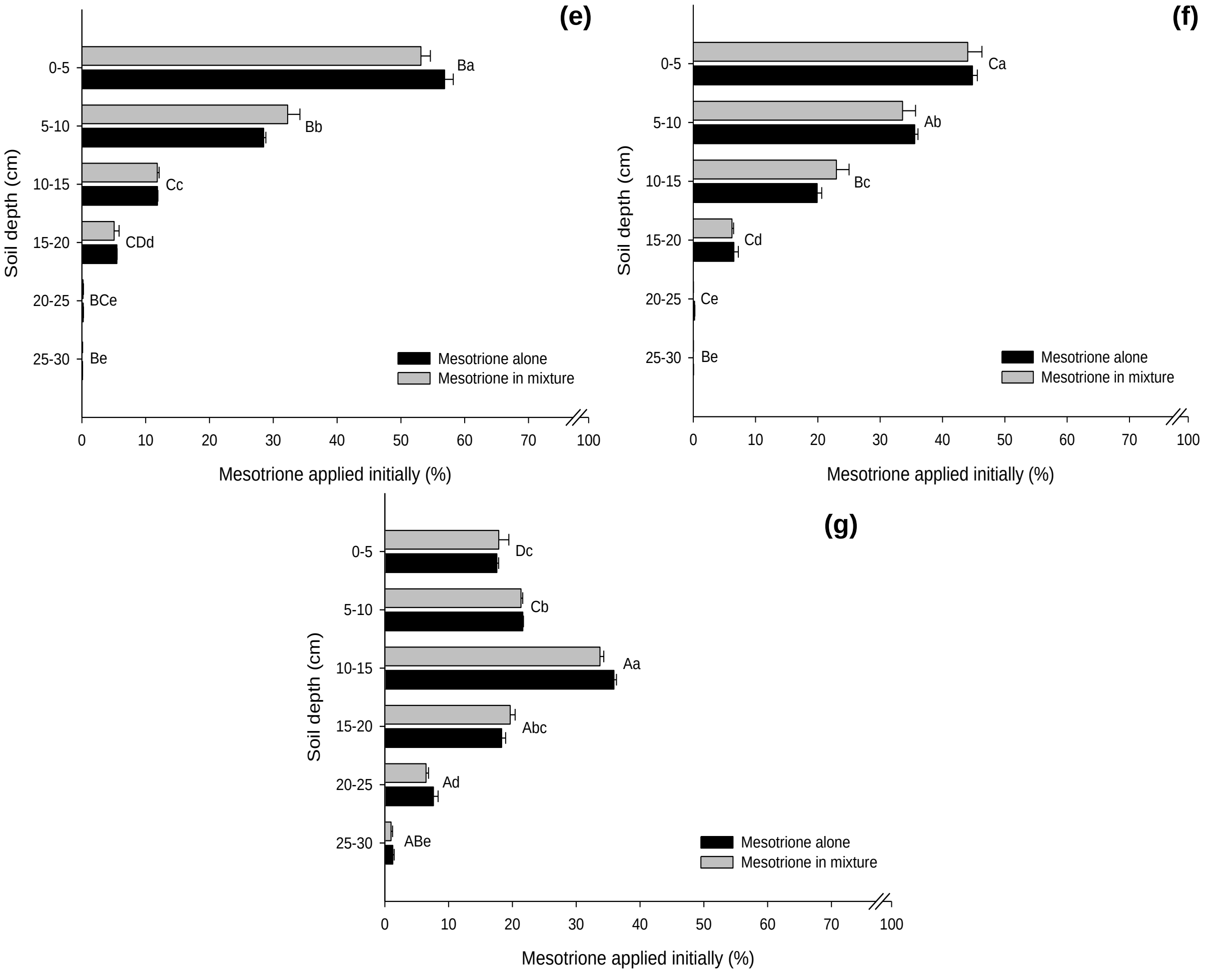
The mass recovery (sum of ^{14}C-mesotrione percentages found in soil depths and leachate) was 94 to 107% and 92 to 108% for the application of the herbicide alone and in mixture, respectively. These values are in accordance with the guidelines of the OECD (2002), which states that recovery experiments with radiolabelled substances can range from 90 to 110%.

All soils displayed observable sorption and leaching of mesotrione. Independent of application form, the leaching of mesotrione in soil is described in ascending order as: BR6 > BR5 > BR7 > BR4 > BR1 > BR2 = BR3. Furthermore, mesotrione residues were found at different concentrations in all soil depths (0–30 cm) in the seven evaluated soils (Figure 4.1). These data are consistent with the mathematical model known as the Groundwater Ubiquity Score (GUS index) (GUSTAFSON, 1989), which is 3.43 for mesotrione and considered a high leachability (MARÍN-BENITO et al., 2014; MILAN et al., 2015). This simple model with preliminary data (DT50 - half-life time and K_{oc} - sorption coefficient OC function) provides information on the leaching potential of mesotrione.

In BR1, leaching of mesotrione was distributed between depths but was found in greater amounts (~37%) in the intermediate layer of 10–15 cm (Figure 4.1a). This was also the case in the same layer of the BR7 sample (~35%) (Figure 4.1g). This may be because despite having different sorption coefficients (K_d), they are both low (BR1 = ~1.05 kg L^{-1} and BR7 = ~0.54 kg L^{-1}) and the desorption of mesotrione is known to be similar in these soils (approximately 25%) (MENDES et al., 2016); therefore, the mesotrione was more available to seepage in the soil. In other soils, mesotrione residues were distributed differently throughout the column.

Figure 4.1 - Percentage ^{14}C-mesotrione applied alone and in mixture with S-metolachlor plus terbuthylazine in glass columns with different depths (0-5, 5-10, 10-15, 15-20, 20-25 and 25-30 cm) and packed with tropical soils, represented by BR1 (Oxisol - Rhodic Hapludox) (a), BR2 (Entisol - Typic Quartzipsamments) (b), BR3 (Entisol - Typic Quartzipsamments) (c), BR4 (Oxisol - Typic Hapludox) (d), BR5 (Oxisol - Typic Hapludox) (e), BR6 (Alfisol - Paleudult) (f) and BR7 (Ultisol - Typic Hapludalf) (g) with simulation 200 mm of water blade for 48 h after application. The horizontal bars associated with each column represent the standard deviation (±SD) of each mean value (*n* = 3). Means followed by the same tiny letter in each depth soil and capital in type soil do not differ by Tukey test ($p<0.05$). DMS (depth) = 3.2916, DMS (soil) = 3.4634 and CV (application form) = 1.60%, CV (soil) = 3.23% and CV (depth) = 12.66%.





The leaching of mesotrione applied in mixture with S-metolachlor and terbuthylazine aged by 24 to 39% of that initially applied to columns of 0–40 cm of sandy soil (PINNA et al., 2014) and can be detected at up to 1.8 m depth silt loam soil, although this was not quantified because of the dose low application (150 g ha^{-1}) and the rapid dissipation (DT50 = 3–7 days) (MILAN et al., 2015).

In BR4, which is a clay soil with high clay content (60.5%) and low pH (6.0) (Table 4.1), the concentration of mesotrione remained high (~74%) in the surface layer (0–5 cm) (Figure 4.2d). Moreover, in BR2 (sandy texture) and BR3 (loamy sand texture) with lower clay contents (0.5 and 12.4%, respectively) and higher pH (7.7 and 7.3, respectively) (Table 4.1) leaching was favoured at all depths not exceeding 5.6% in each layer (Figures 4.1b and 4.1c, respectively). The behaviour of these soils can be explained by the reduced value of K_d (< 0.24 kg L^{-1}) (Mendes et al., 2016), which implies the interaction with the physical and chemical properties of soil on the leaching of mesotrione (Figure 4.2). These results corroborate

Pinna et al. (2014), who found that mesotrione had a weak affinity with the surface of the soil, which was predominantly negatively charged due to the pH range of the analysed soil (5.58 to 6.48) and thus was leached faster.

Rouchaud et al. (2001), reported that mesotrione was leached in a uniform distribution in sandy soil and displayed similar concentrations in all 2 cm layers , up to a depth of 10 cm and that a low amount of waste of mesotrione (6–10 µg kg^{-1} dry soil) reached a depth of 10–15 cm fastest in clay and sandy texture soils compared with loam soil and sandy loam.

Leaching of mesotrione was similar in BR5 and BR6 soil samples, where it remaining concentrated in the surface layer (0–5 cm) with quantities of approximately 55 and 44%, respectively (Figures 4.1e and 4.1f, respectively) but increased with the depth of the soil so that decreased amount of mesotrione and only traces of herbicide (< 0.22%) reached the deepest layers (20–30 cm).

The leaching of herbicides in the soil surface layer (to a depth of 20 cm) is of fundamental importance in agronomic practices, as this is the layer that contains the weed seeds with germination potential, so the chemical control would be more effective.

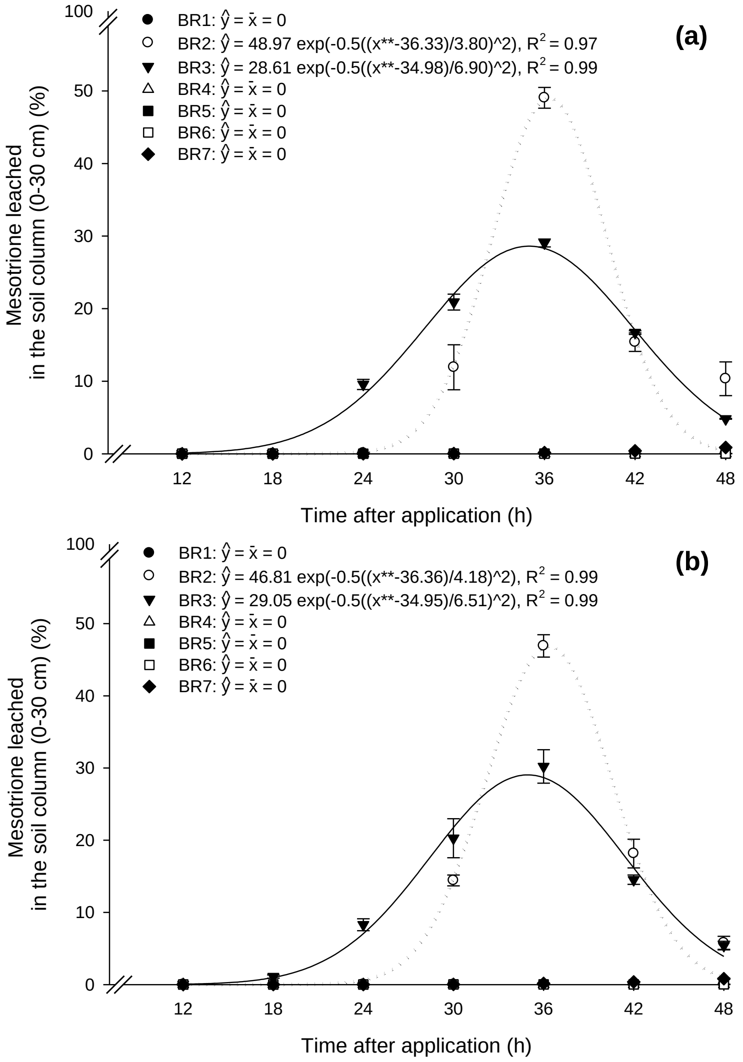
4.3.3. Leachate mesotrione alone and in mixture

In BR2 and BR3 soil samples, the mesotrione was quantified in the leachate at all sampling times as 87.41 and 81.40%, respectively, of the initially applied mesotrione preparation alone (Figure 4.2a). This fact corroborates the small amount of mesotrione found at different depths in the BR2 and BR3 samples, which is justified by the texture (sand and loamy sand, respectively) and pH (7.7 and 7.3, respectively) of these soils (Table 4.1). In the same soils, the mesotrione preparation with S-metolachlor and terbuthylazine displayed a total of 85.69 and 80.56%, respectively, at every sampling time, demonstrating that the combination of chemicals does not influence the leaching of mesotrione (Figure 4.2b). Marín-Benito et al. (2014) detected and quantified, only 19 days after application, mesotrione in leachate at 1 m depth in lysimeters containing clay loam soil, with 32.3 to 43.8% clay, 1.38 to 0.24% OC and 6.68 to 7.87 pH in an irrigated maize monoculture system in Toulosse (France).

In other soils, in both mesotrione preparations, the value of herbicide found in the leachate was minimal (< 1.45%) and showed similar behaviour (Figures 4.2a and 4.2b). However, higher concentrations of mesotrione in these soils were reported at different depths, as described above. According Rouchaud et al. (2001), the mesotrione mainly remains in the topsoil (0–10 cm). This low mobility and penetration depth, in conjunction with mesotrione degradation in soil, explains why there is no movement of the mesotrione to the deeper soil layers.

In BR2 soil, the maximum amount of mesotrione found in the leachate was 48.97 and 46.81% 36.3 h after application of the herbicide alone and in mixture with S-metolachlor and terbuthylazine, respectively (Figures 4.2a and 4.2b, respectively). In BR3, the maximum amount of mesotrione found in the leachate was 28.61 and 29.05% 35.0 h after application of the herbicide alone and in mixture, respectively (Figures 4.2a and 4.2b, respectively). Given the above, we understand that the form of mesotrione application did not interfere in the behaviour in the leachate.

Figure 4.2 - Percentage of leached ^{14}C-mesotrione applied alone (a) and in mixture with S-metolachlor plus terbuthylazine (b) in glass columns (0-30 cm) packed with tropical soils, represented by BR1 (Oxisol - Rhodic Hapludox), BR2 (Entisol - Typic Quartzipsamments), BR3 (Entisol - Typic Quartzipsamments), BR4 (Oxisol - Typic Hapludox), BR5 (Oxisol - Typic Hapludox), BR6 (Alfisol - Paleudult) and BR7 (Ultisol - Typic Hapludalf) with simulation 200 mm of water blade and collected at 12, 18, 24, 30, 36, 42 and 48 h after application. The vertical bars associated with each column represent the standard deviation (±SD) of each mean value (*n* = 3). **p<0.01 by the F test.



Therefore, depending on the type of cultivatable soil with maize, the mesotrione can be easily leached and cause environmental damage as a result of its mobility in the soil profile. Alferness and Wiebe (2002) detected the presence of mesotrione and two metabolites (4-methylsulfonyl-2-nitrobenzoic acid - MNBA and 2-amino-4-methylsulphonylbenzoic acid - AMBA) in groundwater, surface seawater and rivers.

4.3.4. Correlation between leaching and the physical and chemical properties of soils

The mean amount of leaching of mesotrione when applied alone and in mixture with S-metolachlor and terbuthylazine at all depths (0–30 cm) of each soil was correlated with the levels of CM and soil pH, regardless of the application form of the herbicide (Table 4.2). The data presented here are in accordance with the sorption of this herbicide (MENDES et al., 2016), which is inversely proportional to leaching, or if more herbicide is retained in the soil there will be less leaching of this in the soil profile.

Table 4.2 - Pearson correlation of the mean total leaching (%) of the applied mesotrione alone and mixture with S-metolachlor plus terbuthylazine *versus* the physical and chemical properties of tropical soils and equations for calculation of leaching (Lea.).

Mean leaching (%)[a]	Physical and chemical properties[b]				Equation linear[c]
	CEC (mmol$_c$ kg^{-1})	pH (H_2O)	OC (g kg^{-1})	CM (g kg^{-1})	
12.7844	0.5750				Lea. = ns
		-0.8411*			Lea. = 7.7146 – 0.0738 (pH)
			0.6673		Lea. = ns
				0.7517*	Lea. = 10.5832 + 22.3256 (CM)

[a] Corresponds to the mean of the total leaching (*n* = 3) of the seven types evaluated soil at all depths (0-30 cm),

[b] Cation exchange capacity, CEC; potential hydrogen, pH; organic carbon, OC and clay mineral, CM,

[c] ns, correlation is not significant and $^*p<0.05$.

Also, simple linear regression equations were used to generate leaching as a function of the CM content and pH of the soil (Table 4.2). It is important to note that using linear equations to estimate the amount of herbicide leaching using the physical and chemical properties of the soil may be useful in the calculation of the optimal doses for herbicide applications and should improve the predictability of leaching and consequently the mesotrione sorption using mathematical models in soils with similar properties.

The correlation between the average amount of leaching of mesotrione and the pH of the soil was negative (Table 4.2) (i.e., higher pH values in the soil yielded smaller quantities of mesotrione (alone and mixed) leached from the soil depths evaluated). This means that there was greater leaching and mesotrione exceeded 30 cm deep can be found in the leachate, as is the case in this experiment.

Therefore, an increase of soil pH by 1.0 represented a reduction in the average amount of leaching of mesotrione of about 7.64%, regardless of the form of application of the herbicide. Dyson et al. (2002) and Shaner et al. (2012) found that the sorption of mesotrione was negatively correlated with pH. However, Van Der Linden et al. (2008) found that the mobility of mesotrione and its two metabolites (MNBA and AMBA) in soil, increased with the addition of pH.

The data described above can be explained by the fact that mesotrione is a weak acid and has a pKa of 3.1. Thus its molecular form has the ability to donate protons and form negatively charged ions in higher pH environments. Passos et al. (2015) showed that increasing the pH value above the pKa of the herbicide may increase the leaching of sulfentrazone, being a weak acid, as well as mesotrione. In soil pH levels ranging from 6.0 to 7.7, as is the case in this experiment (i.e., above the pKa of the herbicide), the mesotrione molecules are in a dissociated form and thus are more available in the soil solution. Therefore, there is a greater possibility of leaching of mesotrione may contaminate the deeper layers of soil and groundwater even and consequently the surface water.

The correlation between the mean amount of mesotrione leaching and CM content of the soil was positive (Table 4.2), (i.e., higher CM levels in soils resulted in greater amount of mesotrione (alone and in mixture) leached to depths in the evaluated soil (0–30 cm)). This means that less leaching occurred on the ground and that probably mesotrione was retained. Therefore, the addition of 1.0 g kg^{-1} CM on the ground (0.01%) increases, on average, 32.91% of the quantity of leaching of mesotrione, regardless of the form of application of the herbicide. Dyson et al. (2002) and Shaner et al. (2012) found that the sorption of mesotrione was positively correlated with the OC content of the soil.

The lack of a significant correlation between the average leaching of mesotrione and OC contents in this experiment can not be attributed to a narrow range of OC in the soil (0.58 to 27.32 g kg^{-1}) but is more likely due to sorption of mesotrione related to the pH of the soil and to a lesser extent to the OC content, as described by Dyson et al. (2002).

Acidic soils generally have greater sorption capacity of herbicides such as mesotrione and consequently reduced leaching. However, liming as a basic input in maize cultivation can affect mesotrione behaviour, particularly by promoting increased leaching; therefore, the mesotrione can contaminate or intoxicate the crops and poses a high risk of contaminating soil and water.

4.4. Conclusions

The application form of mesotrione alone and in mixture with S-metolachlor and terbuthylazine does not influence the leaching of this herbicide.

Leaching of mesotrione is relatively high in the evaluated tropical soils and thus can present potential groundwater contamination risks and therefore surface water in areas producing maize.

The pH and CM of soils correlate with the leaching of mesotrione, which allows us understand the main parameters responsible for the behaviour of this herbicide in tropical soils. Thus, the use of mesotrione without the prior knowledge of the physical and chemical properties of the topical soil, can result in inefficient weed control and high product leaching risks.

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5. QUANTIFICATION OF THE FATE OF MESOTRIONE APPLIED ALONE OR IN A HERBICIDE MIXTURE IN TWO BRAZILIAN ARABLE SOILS[6]

Resumo

Os efeitos de mesotrione, S-metolachlor e terbuthylazine, aplicado em mistura, na biodegradação do solo permanece pouco estudado. No entanto, misturas de herbicidas tem sido uma prática comum em sistemas agrícolas nos últimos anos. Compreender o destino dos herbicidas aplicados no solo pode ajudar no planejamento de táticas de manejo para o controle de plantas daninhas mais sustentável e eficiente. Portanto, este estudo avaliou o destino do mesotrione isolado e em mistura com S-metolachlor e terbuthylazine quando aplicado a dois solos contrastantes brasileiros que são agricultáveis. Os experimentos de mineralização e de degradação foram conduzidos utilizando ^{14}C-mesotrione isolado ou em mistura. A partir dos dados de laboratório de 49 dias de incubação, o aumento da meia-vida de mineralização do mesotrione foi observado para a mistura de herbicidas, variando de um aumento de 4 dias do solo franco-arenoso para um aumento de 1 dia nos solos de textura argilo-arenosa. A taxa de degradação do mesotrione apresentou um aumento de 2 vezes no solo franco-arenoso em relação ao argilo-arenoso. Dois metabólitos podem ser identificados a partir de degradação do mesotrione, o ácido 4-metil-sulfonil-2-nitrobenzóico (MNBA) e 2-amino-4-metilsulfonil benzóico (AMBA). Índices para a pontuação da ubiquidade nas águas subterrâneas indicou que o mesotrione possui um potencial de lixiviação para ambos os solos. Aplicando mesotrione isolado ou em mistura não influenciou na quantidade de resíduos ligados do mesotrione. No entanto, a taxa de degradação do mesotrione foi influenciada pela textura do solo, independentemente se aplicado isolado ou em mistura. A biotransformação do mesotrione foi relativamente rápida, indicando que este herbicida tem baixa persistência, e, consequentemente, baixo efeito residual sobre as culturas e plantas daninhas, quando presentes em solos semelhantes ao presente estudo.

Palavras-chave: mineralização, mistura de herbicida, degradação, metabólitos.

Abstract

The effects of mesotrione, S-metolachlor, and terbuthylazine, applied in mixture, on soil biodegradation remains insufficiently researched. However, herbicides mixtures have been a common practice in agricultural systems in the last years. Understanding the fate of soil applied herbicides may help on planning weed management tactics towards more sustainable and efficient weed control. Therefore, this study evaluated the fate of mesotrione alone and in mixture with S-metolachlor and terbuthylazine when applied to two contrasting arable Brazilian soils. Mineralization and degradation experiments were conducted using ^{14}C-mesotrione alone or in mixture. From the 49 d laboratory incubation data, increased mineralization half-life of mesotrione were observed for the mixture of herbicides, ranging from a 4 d increase for the sandy loam soil to a 1 d increase in the sandy clay texture soils. Mesotrione degradation rate had a 2-fold increase in the sandy

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[6] MENDES, K.F.; MARTINS, B.A.; REIS, M.R.; PIMPINATO, R.F.; TORNISIELO, V.L. Quantification of the fate of mesotrione applied alone or in a herbicide mixture in two Brazilian arable soils. **Environmental Science and Pollution Research**, Landsberg, v.1, p.1-11, 2017.

loam compared to the sandy clay soil. Two metabolites can be identified from mesotrione degradation, 4-methyl-sulfonyl-2-nitrobenzoic acid (MNBA) and 2-amino-4-methylsulfonyl benzoic acid (AMBA). Indices for the score of ubiquity in groundwater indicated mesotrione possesses leaching potential for both soils. Applying mesotrione alone or in mixture did not influence the amount of bound residues from mesotrione. However, mesotrione degradation rate was influenced by soil texture regardless if applied alone or in mixture. Mesotrione biotransformation was relatively quick, indicating this herbicide has low persistence, and consequently, low residual effect on crops and weeds when present in similar soils to this present study.

Keywords: mineralization, herbicide mixture, degradation, metabolites.

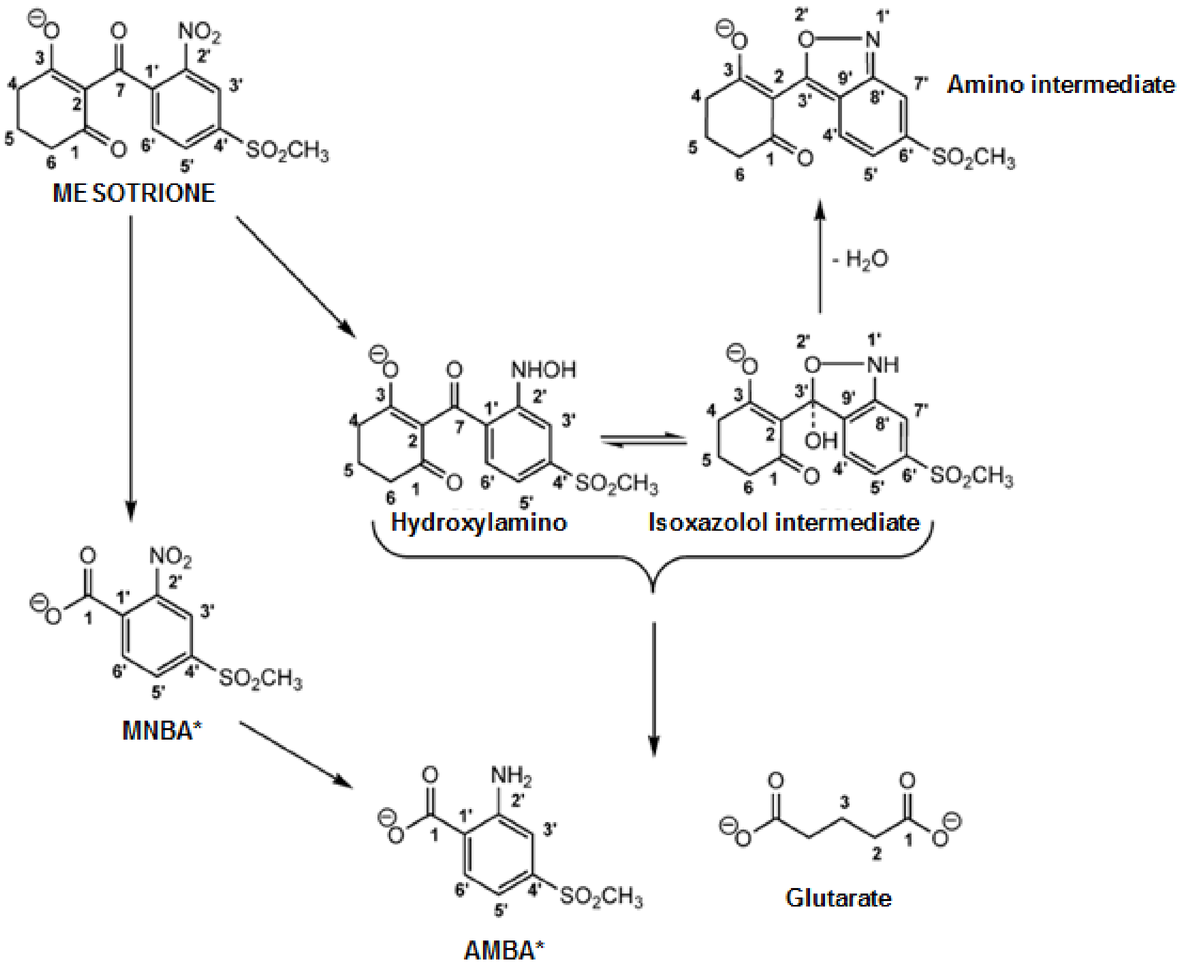
5.1. Introduction

Mesotrione [2-(4-methylsulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione] is a pre- and post-emergent triketone herbicide marketed for the control of broadleaf weed species, primarily in corn (maize) (BEAUDEGNIES et al., 2009). Mesotrione acts by inhibiting the p-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, thereby blocking the production of carotenoid pigments in susceptible plants (ABENDROTHE et al., 2006). Understanding herbicide fate in the environment is important to avoid not only herbicide leaching, but also herbicide carryover issues. Herbicide mineralization and degradation studies elucidate how herbicide behavior in soils is influenced by soil-related variables, including soil texture and herbicide application mode (alone or in mixture).

Mesotrione can be degraded by photolysis at the soil surface and can undergo microbial degradation as it translocates deeper in the soil profile (QUAN et al., 2015). Mesotrione can be completely degraded by some bacteria species, including *Bacillus* sp., *Pantoea ananatis*, *Bradyrhizobium* sp. and *Escherichia coli* (DURAND et al., 2006; 2010; PILEGGI et al., 2012; OLCHANHESKI et al., 2014; CARLES et al., 2016; ROMDHANE et al., 2016). Mesotrione degradation by *Bacillus* sp. is shown in Figure 5.1. Mesotrione is hypothesized to degrade to six main metabolites: 4-methyl-sulfonyl-2-nitrobenzoic acid (MNBA), 2-amino-4-methylsulfonyl benzoic acid (AMBA), hydroxylamino intermediate(the nitro moiety of mesotrione was reduced into hydroxylamine by a nitroreductase), isoxazolol intermediate (a nucleophilic attack of the hydroxyl moiety of the hydroxylamine on the carbonyl can yield a cyclized form of the hydroxylamine), amino intermediate (a deshydratation reaction leads to a benzisoxazole derivative from isoxazolol intermediate) and glutarate (ALFERNESS; WIEBE, 2002; DURAND et al., 2010). Compared to the parent compound mesotrione, its metabolite AMBA has been found to possess greater toxicity in selected microorganisms, including *Tetrahymena pyriformis* and *Vibrio fischeri* (BONNET et al., 2008).

Because mesotrione is a weak acid, pH can influence its fate. For example, DYSON et al. (2002) found that as pH increased, both sorption and degradation time decreased. In the same study, there was a positive correlation between mesotrione sorption and degradation, reflecting the potential these variables have on assessing mesotrione environmental impact.

Figure 5.1 - Mesotrione degradation pathway by *Bacillus* sp. 3B6. *Mesotrione transformation products also detected in the present study. Source: Durand et al. (2010).



In addition to soil properties, studies have found that whether a herbicide is applied alone or as part of a multi-herbicide formulation may also affect degradation in soils (BONFLEUR et al., 2011). Mesotrione is often used in combination with S-metolachlor and terbuthylazine in order to increase the spectrum of weed control. However, little is known about the effects of the mixture mestrione + S-metolachlor + terbuthylazine on the behavior of mesotrione in the environment, especially in tropical regions of Brazil.

Mineralization studies using mixtures of herbicides are fundamental for improving stewardship guidelines. In the agricultural and environmental setting, herbicides are frequently applied in mixture with different herbicides, in varying soil types, and weather conditions. Therefore, the effects of herbicide mixtures on herbicide degradation processes in the soil must be understood in order to make herbicide use more efficient and environmentally sustainable. This study evaluated the fate of mesotrione applied alone or in mixture with S-metolachlor and terbuthylazine when applied to two soil types from Brazil.

5.2. Materials and Methods

5.2.1. Soil sampling and preparation

Soil samples were collected from the surface layer (0-10 cm) without superficial straw at two different maize producing areas (Alfisol – Paleudult, S 22°42’34”, W 47°37’18” and Ultisol - Typic Hapludalf, S 22°42’52”, W 47°37’10”) in Piracicaba, Sao Paulo State, Brazil. Samples from soils that had not been treated with the herbicides mesotrione, S-metolachlor and terbuthylazine for the past 2 years. Soils samples were air-dried for 7 d, sieved in a 2 mm soil screen, and the physicochemical properties of the samples and the classification of the soils are shown in Table 5.1. Soil texture was determined using the hydrometer method. Soil pH was measured in a 1:2 soil/deionized water mixture. Soil OC content was determined by dry combustion at 900°C and measurement of CO_2 evolution using a C/N Analyzer (VarioMAX; Elementa Americas, Inc., Mt. Laurel, NJ). The soil moisture content (*w*) was 0.0762 and 0.0163 kg kg^{-1} for Alfisol – Paleudult and Ultisol - Typic Hapludalf, respectively.

Table 5.1 - Physicochemical properties of soils (0-10 cm of depth).

Soil^a	pH (H_2O)	K	Ca^{2+}	Mg^{2+}	H + Al	BS	CEC
					(mmol$_c$ kg^{-1})		
NVef	6.4	11	51	26	41	88	129
PVAd	6.9	1	18	7	29	26	55
Soil^a	P (mg kg^{-1})	V (%)	OC (g kg^{-1})	sand	clay	silt	texture class
					(g kg^{-1})		
NVef	18	68	18.02	466	376	158	sandy clay
PVAd	15	47	5.23	816	151	33	sandy loam

^{a} According to Soil Taxonomy and Brazilian Soil Science Society (EMBRAPA, 2013).
NVef = Nitossolo Vermelho eutroférrico (USDA: Alfisol – Paleudult; sandy clay);
PVAd = Argissolo Vermelho-Amarelo distrófico (USDA: Ultisol - Typic Hapludalf; sandy loam);
H + Al: potential acidity; BS = base saturation; CEC = cation exchange capacity; V = base saturation levels; OC = organic carbon.
Source: Soil Science Department - ESALQ/USP, Piracicaba, SP, Brazil.

5.2.2. Chemicals

Analytical standards of mesotrione, S-metolachlor and terbuthylazine with 99.9, 98.2% and 98.8 purity, respectively, were purchased from Sigma Aldrich (St. Louis, MO, USA). ^{14}C-labeled [cyclohexane-2-^{14}C] mesotrione (Izotop, Budapest, Hungary) with a radiochemical purity of 98.4% and specific activity of 3.45 MBq mg^{-1} was used in the experiments.

Two mesotrione solutions were prepared, one containing solely mesotrione and a second one containing a mixture of mesotrione (6.97%) with S-metolachlor (58.15%) and terbuthylazine (34.88%) corresponding to the commercial herbicide mixture Lumax®, recommended in maize. The mesotrione solution was prepared in 0.01 M $CaCl_2$ using both unlabeled and ^{14}C-mesotrione to give a total concentration of 31.25 mg L^{-1} (activity ≈ 113.45 Bq mL^{-1}). The herbicide mixture was also prepared in 0.01 M $CaCl_2$ using the unlabeled standards of S-metolachlor and terbuthylazine mixed with ^{14}C-mesotrione. The mixture contained 31.25 mg L^{-1} of mesotrione (activity ≈ 113,450 Bq mL^{-1}), 260.50 mg L^{-1} of S-metolachlor and 156.25 mg L^{-1} of terbuthylazine.

5.2.3. Mineralization and degradation experiments

The experiments were conducted according the methods established by the Organization for Economic Co-Operation and Development (OECD, 2002), in a completely randomized 2x2 factorial design with 3 replicates. The two factors were soil type (sandy clay or sandy loam) and mesotrione application mode (alone or in mixture with S-metolachlor and terbuthylazine). Each experimental unit consisted of one 250 mL biometer culture flask (Fisher C-4443-250) equipped with a side tube which was used to contain the 10 mL of 0.2 M sodium hydroxide (NaOH) as the carbon dioxide (CO_2) trap.

Dry soil (50 g) was adjusted to 75% of field capacity (COSTA, 1983), placed in the biometer flask and pre-incubated in the dark at 20 ± 2°C for 7 d. Herbicide solutions (200 µL) were applied to each experimental unit, corresponding to the maximum recommended dose of each herbicide (assuming soil bulk density = 1200 kg m^{-3}, incorporation depth = 0.10 m). Soil moisture content was maintained throughout the incubation period by weighing the flasks every seven days and adjusting with distilled water when necessary.

Mesotrione mineralization was quantified based on the amount of $^{14}CO_2$ trapped in the 0.2 M NaOH solution from each flask. NaOH solutions were analyzed by liquid scintillation spectrometry (LSS) with a Tri-Carb 2910 TR counter (LSA PerkinElmer). Two 1 mL aliquots of NaOH solution were taken at 7, 14, 21, 28, 35, 42 and 49 days after herbicide application. At each evaluation time, fresh NaOH solution was placed again in the side tube of each flask. The entry of atmospheric CO_2 into the flasks was prevented by using "lime soda" filters.

Degradation was determined by measuring the remaining ^{14}C-mesotrione in the soil at 0, 7, 14, 21, 28, 35, 42 and 49 days after application. Soils were transferred from the flasks to Teflon tubes (200 mL) with 20 mL of 0.05 M ammonium hydroxide (NH_4OH) and 80 mL of acetone added to each tube. Samples were shaken (horizontally) at 200 rpm for 30 min, centrifuged at 4000 rpm for 15 min, and the supernatant was collected and transferred to a 5 mL glass vial (DYSON et al., 2002). The extraction procedure was repeated, but with addition of 10 mL 0.05 M NH_4OH and 40 mL of acetone. The supernatants were combined, and a 1 mL-aliquot was transferred to a liquid scintillation vial containing 10 mL of scintillation cocktail. Radioactivity was measured by LSS. The remaining extract solution was evaporated under vacuum at 40°C and 1 mL aliquots of the concentrated extract was again analyzed for radioactivity by LSS. The remaining concentrated extracted was analyzed for mesotrione and potential mesotrione metabolites by thin layer chromatography (TLC) (described below).

Extracted soil samples were dried at 40°C and then ground by a mechanical mill. Subsamples of each soil were weighed out in triplicate (0.2 g) and biologically oxidized (OX500, RJ Harvey Instrument Corporation) to determine the amount of non-extractable herbicide [^{14}C-bound residues]. The oxidizer's efficiency was calculated prior to sample combustion in order to correct any recovery error. The mass balance of radioactivity was then calculated.

5.2.4. Thin layer chromatography

Thin layer chromatography (TLC) was performed according to US Environmental Protection Agency method 507 (EPA, 1998; FRIED; SHARMA, 1999). Aliquots of the concentrated extracts (0.1 mL) were applied to TLC plates (60F254, EMD Millipore) with a micro syringe. TLC plates were placed in a chamber saturated with 100 mL of acetronitrile:water (70:30). The analytical standard of each herbicide in question (0.1 mL) was also spotted concurrently.

TLC plates were then allowed to dry for a subsequent radio scan (Packard, Cyclone - Perkin-Elmer) for detection of the applied spots in the plates. For each analysis, the solvent front and distances moved by mesotrione and its metabolites were measured and used to calculate for the retention factor (Rf).

5.2.5. Mesotrione mineralization and degradation model

Data of $^{14}CO_2$ produced and ^{14}C-mesotrione present in extractable residues were fit to a first order kinetic model: $C = C_0 e^{-kt}$, where C is mesotrione concentration at time t (%); C_0 is mesotrione concentration at time zero (%); k is a mineralization rate constant (d^{-1}); and t is the incubation time (days). Mineralization and degradation half-life times (MT50 and DT50), defined as the time required for 50% of the applied herbicide to be mineralized to CO_2 or degraded, were calculated as follows: MT50 or DT50 = ln 2/k (PICTON; FARENHORST, 2004).

5.2.6. Environmental fate implications

To determine the correlation between mesotrione sorption and degradation coefficients, the Groundwater Ubiquity Score (GUS) index (a simple index of potential leaching) was calculated using measured ‘‘paired values’’ of sorption coefficient (K_{oc}) and DT50 from two soils, according to the equation: GUS = $\log_{10}$ (DT50) x [4 – $\log_{10}$ (K_{oc})] (GUSTAFSON, 1989).

5.2.7. Statistical analysis

Mesotrione mineralized, extractable (parent herbicide and their metabolites), bound residues and GUS indexes data were subjected to analysis of variance (ANOVA) to verify the interaction between soil type and mesotrione application mode. But just mesotrione mineralization (CO_2 production) and degradation (C_{14}-mesotrione extractable on the soil) data when significant, averages were compared by Tukey's honest significant difference (HSD) test ($p<0.05$). Figures were plotted using Sigma Plot (version 10.0 for Windows, Systat Software Inc., Point Richmond, CA).

5.3. Results and Discussion

5.3.1. Soil analyses

Laboratory analyses confirmed the two soils were of contrasting texture (Table 5.1). These two types of soil (distance between the two sites is <2 km) are common in the studies Southeast region of Brazil (LEAL et al., 2013).

5.3.2. Mesotrione mineralization

The In this study, mesotrione mineralization was determined by quantifying the $^{14}CO_2$ produced. Radiolabeled carbon dioxide, as the ^{14}C-mesotrione mineralization product, could be formed by several pathways, including the involvement of the oxidative opening of mesotrione cyclic structure or aromatic metabolites such as MNBA and AMBA, leading to the formation of short chain carboxylic acids, and then of CO_2 and inorganic ions.

Mineralized ^{14}C-mesotrione accumulation, represented by $^{14}CO_2$ accumulation, was lower in the sandy loam (65-70%) than in the sandy clay soil (85-83%) ($p<0.05$) (Figure 5.2). For both soil types, when mesotrione was applied alone, rather than in mixture, this herbicide mineralized faster ($p<0.05$) (Table 5.2; Figure 5.2). Likewise, mesotrione mineralization half-life time (MT50) increased when this herbicide was in mixture with S-metolachlor and terbuthylazine compared to when mesotrione was applied alone. The decrease in mesotrione mineralization rate by the soil microorganisms when this herbicide is applied in mixture may be due to the presence of additional food sources in the herbicide mixture available for the microbiota, other than mesotrione (S-metolachlor and terbuthylazine in this case). In our study, mesotrione degradation can be attributed to the soil microorganisms, given we measured the $^{14}CO_2$ produced directly from ^{14}C-mesotrione mineralization. However, assessing microbial activity of the soils would be a valuable data.

For both soils, the slower mineralization rate when mesotrione is applied alone leads to longer MT50s, which could potentially provide prolonged weed control or additional opportunities for mesotrione leaching from the soil system. However, values for mesotrione mineralization observed in this study are considered to have relatively small environmental impact, being still within the range of a non-persistent herbicide (PPDB, 2016).

Table 5.2 - $^{14}CO_2$ accumulated at the 49 d of incubation (%) and parameters of the first order kinetics (k and MT50) according $^{14}CO_2$ released until at the 49 d of incubation of the applied ^{14}C-mesotrione alone and as a mixture with S-metolachlor + terbuthylazine in Alfisol – Paleudult (sandy clay) and Ultisol - Typic Hapludalf (sandy loam).

Soil	Mesotrione	Parameter					
		$^{14}CO_2$ (%)		k (d^{-1})	MT50 (days)		R^2
sandy clay	alone	85.95 $\pm$ 1.27[a]	Aa*	0.0401 $\pm$ 0.0018	17.31 $\pm$ 0.80	Bb	0.99
	mixture	84.19 $\pm$ 0.73	Ba	0.0377 $\pm$ 0.0009	18.42 $\pm$ 0.46	Ab	0.99
sandy loam	alone	69.96 $\pm$ 0.61	Ab	0.0245 $\pm$ 0.0004	28.24 $\pm$ 0.48	Ba	0.99
	mixture	65.24 $\pm$ 0.92	Bb	0.0216 $\pm$ 0.0005	32.15 $\pm$ 0.79	Aa	0.99
DMS		1.41			1.01		
CV (%)		1.20			2.73		

[a] The numbers correspond to the mean ($n = 3$) followed by $\pm$ standard deviation.

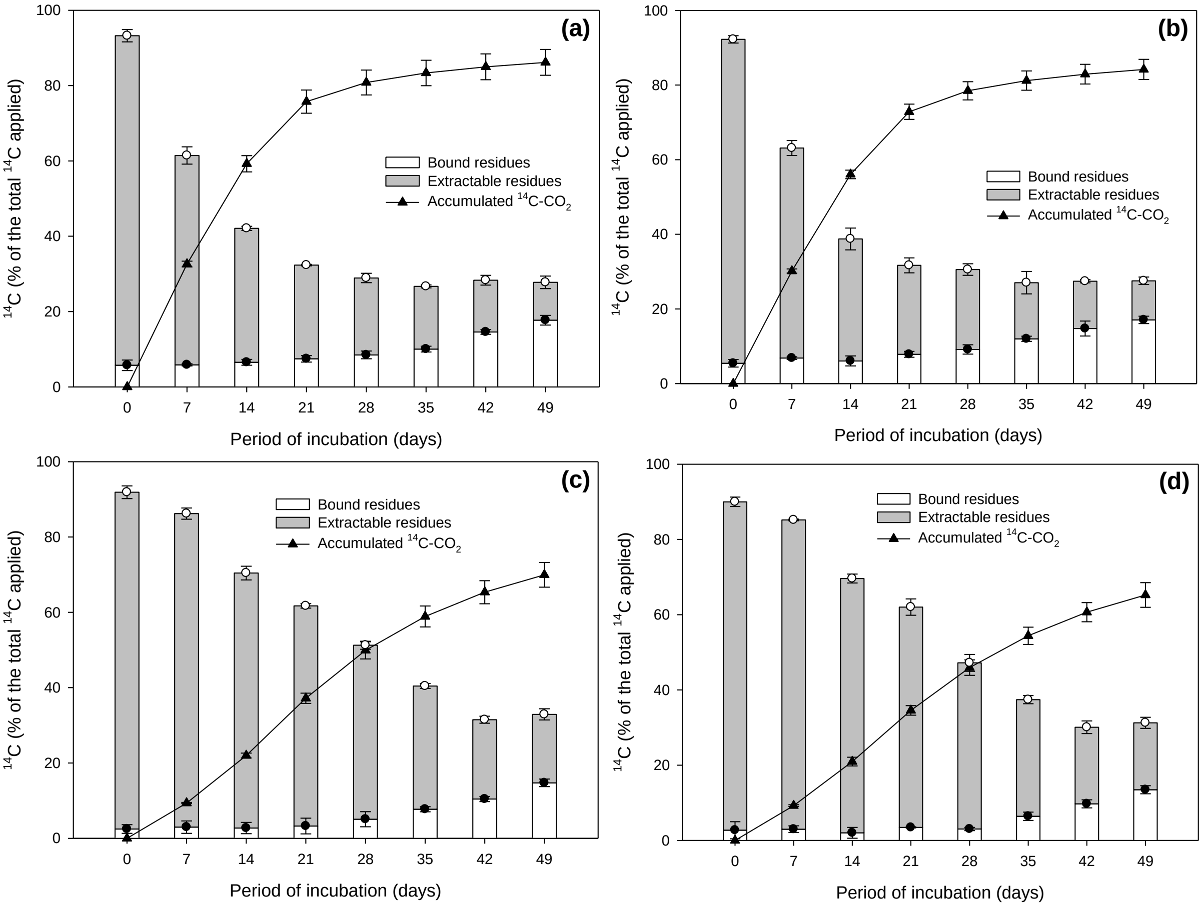
* Means followed by the same lowercase letter at each soil and same capital letter at the herbicide alone/mixture does not differ by Tukey test ($p<0.05$).

5.3.3. Extracted residues

Total extractable mesotrione amounts decreased over the incubation time in both soil types, ranging from 77 to 12% and 89 to 18% in the sandy clay and sandy loam soils, respectively. When mesotrione was applied alone, the amount extracted was 8-9% greater than when this herbicide was applied in mixture with S-metolachlor and terbuthylazine ($p<0.05$), regardless of the soil type (Figure 5.2).

The greater mesotrione mineralization observed in the sandy clay compared to the sandy loam soil ($p<0.05$) most likely contributed for the reduction in the amount of extractable residue in the sandy clay soil. The reduction in the amount of extractable residue in the sandy clay compared to the sandy loam soil could be also due to the formation of more bound residues in the latter soil compared to the sandy loam one.

Figure 5.2 - Distribution of ^{14}C-mesotrione applied alone (a and c) and in mixture with S-metolachlor + terbuthylazine (b and d) in soil Alfisol – Paleudult, sandy clay (a and b) and Ultisol - Typic Hapludalf, sandy loam (c and d) among extractions with solvent, mineralized ^{14}C-CO$_2$, and bound residues (%) as a function of incubation time (49 d) in soil samples. Vertical bars represent standard deviations (± SD) of means (*n* = 3).



5.3.4. Bound residues

The formation of bound residues and mesotrione metabolites also increase over the incubation period in both soil types (Figure 5.2). After 49 d, more bound residues (~18%) were detected in the sandy clay compared to the sandy loam soil (~14%).

When mesotrione was applied alone, bound residues was 3-5% greater than when this herbicide was applied in mixture than alone ($p<0.05$), regardless of the soil type (Figure 5.2). In general, the formation of bound residues remained constant between 21 and 49 d for all studies. Apparently, formation of bound residues from mesotrione is not associated with mesotrione sorption in the soil because this herbicide exhibited similar retentions in soils with contrasting textures whether applied alone or in mixture with S-metolachlor and terbuthylazine (MENDES et al., 2016). The greater bound residues formation in the soil when mesotrione was applied in mixture rather than alone must explain the lower amounts of extractable residues found in the mixture treatment compared to mesotrione alone treatment. Because neither soil type nor application mode influenced mesotrione sorption in the soils with different physico-chemical properties (MENDES et al., 2016), we assume that formation of bound residues from mesotrione does not relate to its sorption in the soils studied.

According to Führ et al. (1998), bound residues represent compounds that persist in the matrix in their original form or as metabolites after extraction. It is important that the extraction method does not substantially alter the compounds or the matrix structure. For studies using ^{14}C-labeled molecules, the operational definition of bound residues would be all radioactivity not extracted from the soil and recovered only after combustion in an oxidizer. Bound residues (i.e. the remaining radioactivity as $^{14}CO_2$) are then quantified by liquid scintillation counting (BARRIUSO et al., 2008). This method, however, does not allow the distinction between parent material and metabolite species present as bound residues.

Alonso et al. (2015) reported that the amount of bound residues for indaziflam herbicide and their metabolites increased at a higher rate during the first 14 d of the incubation period, thereafter stabilizing or decreasing throughout the rest of the incubation. According to Koskinen and Harper (1990), a herbicide may be initially retained by fast mechanisms of low binding energy and subsequently be converted into more stable ones of high binding energy over time. Thus the increase of bound residues with time is likely due to greater physical and chemical interactions of herbicide molecules with the soil.

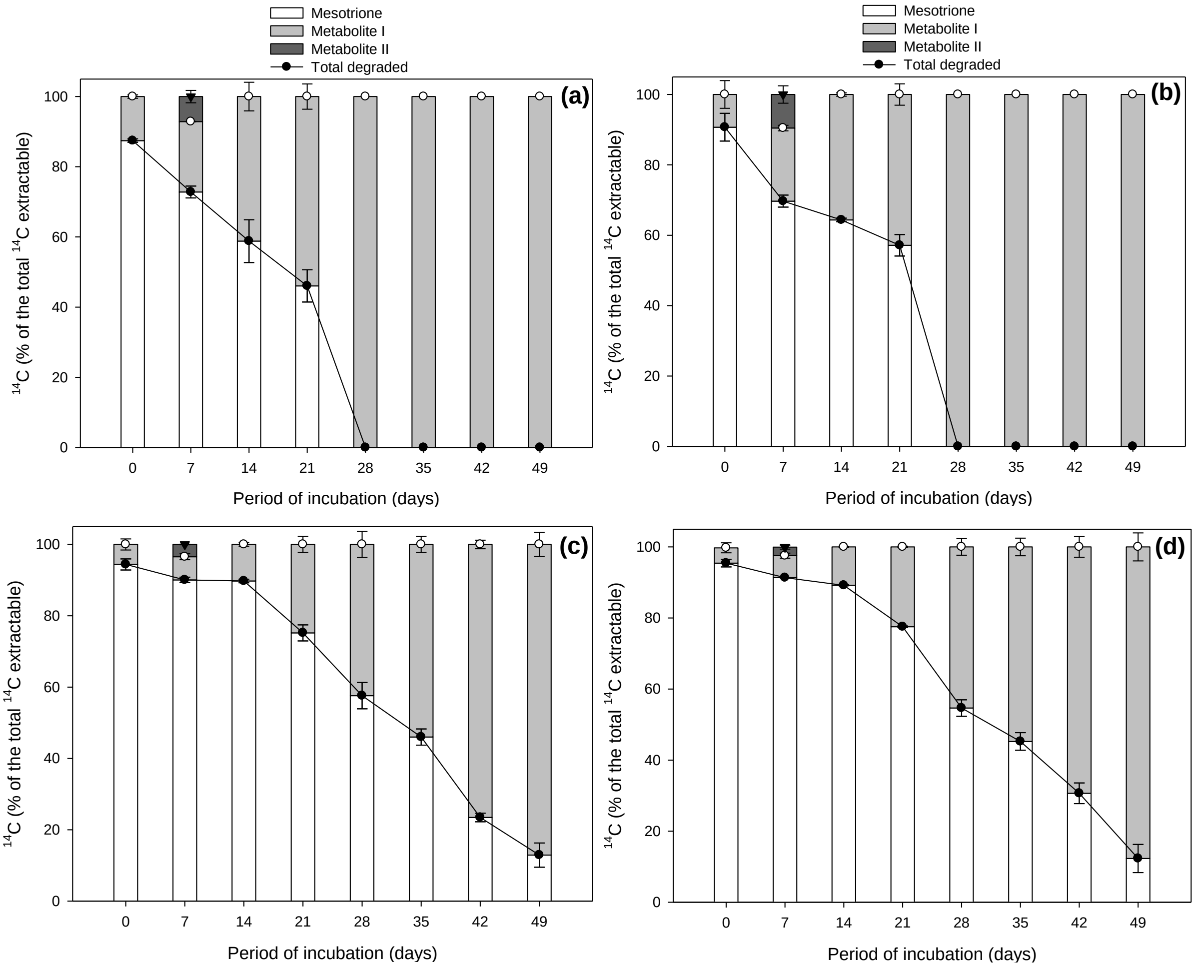
5.3.5. Mass balance

Mass balances for ^{14}C-mesotrione (mineralized, extractable, and bound residues to soil) averaged for the eight sampling times were quantified (Figure 5.2). Recovery of ^{14}C was 93 to 109% in the sandy clay and 91 to 104% in the sandy loam soil. These data are consistent with the guidelines by Organization for Economic Co-Operation and Development (OECD, 2002), which allows a mass balance of radioactivity between 90 and 110%.

5.3.6. Mesotrione degradation as affected by soil type and application mode

Mesotrione degradation was analyzed by measuring ^{14}C amounts present on the extractable residues from the soils. There was no interaction between soil type and herbicide application mode when mesotrione degradation was analyzed ($p>0.05$). Therefore, mesotrione degradation was similar in both soils, whether applied alone or in mixture (Table 5.3; Figure 5.3).

Figure 5.3 - Formation of degradation products of ^{14}C-mesotrione extractable applied alone (a and c) and in mixture with S-metolachlor + terbuthylazine (b and d) in soil Alfisol – Paleudult, sandy clay (a and b) and Ultisol - Typic Hapludalf, sandy loam (c and d) as a function of incubation time (49 d) in soil samples. Vertical bars represent standard deviations (± SD) of means (*n* = 3).



A lag phase in mesotrione degradation was observed for the sandy loam soil because mesotrione mineralization was slower in this soil compared to the sandy clay one. Thus, more mesotrione was available for microbial degradation in the sandy loam soil than in the sandy clay soil during the lag phase (Figures 5.2 and 5.3).

In the sandy clay soil, a decrease from 90 to 46% of ^{14}C present in the extractable residues was observed between 0 to 21 d. Complete mesotrione degradation by the soil microorganisms was observed by 28 d after application in the sandy clay soil, where significant increases in ^{14}C were no longer detected. In a study using Brazilian soils of different textures, mesotrione sorption was positively correlated with organic carbon content (OC) in the soil (MENDES et al., 2016). These authors found that mesotrione sorption was greater in soils with greater OC. Therefore, it seems reasonable to assume that the available mesotrione from the sandy clay soil solution was all degraded because another portion of the herbicide was sorbed into the soil colloidal fraction (clay and organic matter), which occurred in greater amounts in the sandy clay compared to the sandy loam soil.

This did not occur in the sandy loam soil, which had 95-12% of mesotrione present between 0 and 49 d of incubation (Figure 5.3). The difference in mesotrione present in the soil solution over time between the two soil types may be attributed to the difference in the OC content and pH of the soils, which are directly related to the difference in microbial communities' type and amount.

Table 5.3 - ^{14}C degraded present on the extractable residues at the 49 d of incubation (%) and parameters of the first order kinetics (k and DT50) according ^{14}C-mesotrione until at the 49 d of incubation of the applied ^{14}C-mesotrione alone and as a mixture with S-metolachlor + terbuthylazine in Alfisol – Paleudult (sandy clay) and Ultisol - Typic Hapludalf (sandy loam).

Soil	Mesotrione	Parameter					
		^{14}C (%)		k (d^{-1})	DT50 (days)		R^2
sandy	alone	97.01 ± 0.56[a]	Ab*	0.0565 ± 0.0024	12.27 ± 0.54	Ab	0.93
clay	mixture	99.16 ± 4.06	Ab	0.0535 ± 0.0024	12.96 ± 0.58	Ab	0.91
sandy	alone	107.34 ± 0.92	Aa	0.0259 ± 0.0006	26.82 ± 0.66	Aa	0.92
loam	mixture	107.96 ± 1.01	Aa	0.0255 ± 0.0011	27.23 ± 1.21	Aa	0.93
DMS		4.25			1.56		
CV (%)		2.10			4.01		

[a] The numbers correspond to the mean (*n* = 3) followed by ± standard deviation.

* Means followed by the same lowercase letter at each soil and same capital letter at the herbicide alone/mixture does not differ by Tukey test ($p<0.05$).

Mesotrione is easily degraded, so it is not stable in the environment, being detected in soil and water in short periods of time after its application (BARCHANSKA et al., 2012). This herbicide can be degraded by abiotic and biotic processes. *Bacillus* sp. strains (DURAND et al., 2006; BATISSON et al., 2009; CARLES et al., 2016) and *Bradyrhizobium* sp. SR1. (ROMDHANE et al., 2016) isolated from agricultural soil have been shown to completely and rapidly degrade mesotrione.

As opposed to application mode, soil type influenced mesotrione degradation, which was twice the rate in the sandy loam soil (DT50 = 12) as compared to the sandy clay soil (DT50 = 27 d) (Table 5.3). The degradation rate constants (k), were approximately 0.055 and 0.025 per day in the sandy clay and sandy loam soils, respectively. The total amount of mesotrione found in this study was greater in the sandy loam soil during all time periods compared to the sandy clay soil (Table 5.3). Similarly, DT50 for mesotrione degradation was 9.7 d in a Spanish sandy loam soil (10.7% clay), which after being sterilized, presented DT50 of 24.3 d (JUAN et al., 2015a). These authors suggested that the increase in mesotrione DT50 was mainly due to biodegradation.

Primary biodegradation can involve structural changes in the herbicide through oxidation, reduction or loss of a functional group, or involve several sequential reactions, resulting in the loss or modification of the herbicide activity. In a study, mesotrione DT50 was below 3 d across four soils with active microbial activity and below 28 d when the same soil was irradiated (SHANER et al., 2012). As well as in our study, mesotrione degradation was relatively rapid.

It is vital to understand that DT50 of herbicides is usually less than MT50, as it was observed in our experiment, in which mesotrione is degraded from the moment it is transformed by physical, chemical and biological processes into metabolites, and in which this herbicide is completely mineralized to water, CO_2 and inorganic compounds.

The DT50/MT50 ratio was greater in the sandy clay soil compared to the sandy loam soil, with values of 1.41 and 1.42 for mesotrione alone and in mixture, respectively, and in sandy loam was lesser with values of 1.05 and 1.18 to mesotrione applied alone and in mixture, respectively. A difference in microbial community structure between the sandy clay and sandy loam soil, with a preponderance of the mesotrione degrading microorganisms in the sandy clay soil, could be the underlying cause of why mesotrione DT50/MT50 ratio was greater in the sandy clay compared to the sandy loam soil. Again, evaluating the microbial composition of the soils would be a valuable piece of data. For example, Joly et al. (2012) observed that mesotrione degradation time and deleterious effects on microbial communities were increased when low quantities of the mixture mesotrione + S-metolachlor were applied compared with ten times the rate of each herbicide alone. Therefore, the combined effects of mesotrione with S-metolachlor may affect the microbial community responsible for mesotrione degradation differently than the isolated products, affecting mesotrione metabolism and environmental fate.

Given the above, the ideal is that herbicides with degradation experiments are associated with mineralization to effectively understand the transformation of the molecules in the soil and to recommend appropriate management for weed control in crops, minimizing environmental impacts.

5.3.7. Formation of mesotrione metabolites

No interaction was found between application mode and soil type on mesotrione metabolite formation ($p>0.05$). During the degradation process of mesotrione applied alone and in mixture, there was the formation of two metabolites evaluated in both soils, with similar behavior (Figure 5.3). Mesotrione was transformed to metabolite I and II in greater proportion in the sandy clay compared to the sandy loam soil for all time periods. After 28 d of incubation, 100% of the total ^{14}C had been already extracted in the sandy loam soil. In addition, metabolite I formation gradually increased over time, with 87% of metabolite I present after 49 d of incubation (Figure 5.3). Metabolite II formation took place after 7 d of incubation, with greater amount formed in the sandy clay soil (10%) compared to the sandy loam soil (4%) (Figure 5.3). This might happen because mesotrione DT50 in the sandy clay soil is lower than in the sandy loam soil, that is, the degradation process is accelerated, resulting in increased formation of metabolites.

Metabolism of mesotrione is first initiated through hydroxylation of the compound (ARMEL et al., 2005). After hydroxylation, the cyclohexane and phenyl groups of these hydroxy metabolites are then hydrolytically split, forming two immobile and nonherbicidal metabolites, MNBA and AMBA (Figure 5.1).

TLC analysis of extracts from ^{14}C-mesotrione applied in the weed revealed three major bands of radioactivity (ARMEL et al., 2005), similar to data found in this study (Figure 5.3). The parent mesotrione was identified with a retention factor (Rf) value of 0.7. MNBA (metabolite I) and AMBA (metabolite II), can be identified with corresponding Rf values of 0.4 and 0.6, respectively. These values corroborate with the reported by Armel et al. (2005) and Barchanska et al. (2015). Rf values of mesotrione and its metabolites were confirmed by applying a dilution stock of ^{14}C-labeled mesotrione, AMBA, and MNBA in addition to the soil extracts on the TLC plates. However, the hydroxy metabolite precursors and AMBA were not sufficiently separated by the TLC system, so the inactive metabolites at Rf value 0.6 are a combination of AMBA and the hydroxy metabolite precursors (the hydroxylamino derivative in equilibrium with the cyclized isoxazolol intermediate, and amino intermediate) (CARLES et al., 2016). AMBA is known to be formed from MNBA and to have lower persistence in soils compared with MNBA (DURAND et al., 2010) (Figure 5.3). In a study conducted by Pesticide Properties Database (PPDB, 2016), MNBA DT50 was 7.5 d, whereas AMBA DT50 was 3.2 d, and were considered non-persistent compounds in the soil.

The two main metabolites (MNBA and AMBA) formed from mesotrione biodegradation in the soil in this study were also found by Crouzet et al. (2010), Durand et al. (2010) and Juan et al. (2015b). AMBA was detected mainly in the soil without addition of organic compounds, which indicates that mesotrione was not completely mineralized. According to results by Juan et al. (2015b), AMBA was considered to be an end product of mesotrione biodegradation in the soil. These authors stated also that MNBA metabolite was more frequently detected and considered to be an AMBA precursor. Another study has found AMBA to be present in soils applied with mesotrione alone in two concentrations (1.5 or 10 mM) (DURAND et al., 2010). In the same study, AMBA detection occurred after 3.5 h.

5.3.8. Mesotrione leaching potential

Mesotrione potential on achieving water resources was determined based on its DT50 and K_{oc}. GUS indices values not interacted significantly between soil type and herbicide application mode ($p>0.05$). For mesotrione applied alone and in mixture, GUS indices were 2.34 and 2.44 for the sandy clay soil, respectively.

The same index for both application modes in the sandy loam soil were 2.85 and 2.84, respectively. GUS indexes above 2.8 indicate that a herbicide is easily leached and values below 1.8 indicate that the same herbicide is "no leachable". There is a transition range between 1.8 and 2.8, where the herbicide can be leached in favorable conditions. Based on mesotrione GUS indices from this study, this herbicide can be considered potentially leachable when applied to a sandy clay soil and leachable when applied to a sandy loam soil.

GUS index for mesotrione averaged 2.20 (DYSON et al., 2002). This features the mesotrione within transition range, where can be leached. Chaabane et al. (2008) reported GUS index varying from 1.9 to 3.2, and considered mesotrione moderately or easily leached. A more detailed and complete approach to determine herbicide leaching potential in a soil is the conduction of experiments in which the herbicide is applied into the soil inside columns or lysimeters.

5.4. Conclusions

Study on the quantification of the fate of herbicides alone and in mixture in the soil is of outmost importance, considering the number of herbicides mixture that are currently used in different soil types and climatic conditions. Further studies of the fate of herbicides mixture in soil may provide insight into the mechanism of degradation of other herbicides and may suggest ways in which mixture may affect to microbial activity in the soil. Mesotrione biotransformation was relatively quick, indicating this herbicide has low persistence, and consequently, low residual effect on crops and weeds when present in similar soils to this present study.

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6. GLUCOSE MINERALIZATION IN SOILS OF CONTRASTING TEXTURES UNDER APPLICATION OF S-METOLACHLOR, TERBUTHYLAZINE, AND MESOTRIONE ALONE AND IN A MIXTURE[7]

Resumo

A adaptação microbiana pode ocorrer na superfície do solo de uso agrícola exposto a herbicidas. No entanto, pouco se sabe sobre os efeitos da mistura de herbicidas no solo, especialmente em regiões tropicais como no Brasil. O objetivo deste estudo foi avaliar a mineralização da glicose em solos de texturas contrastantes (argila-arenosa e franco-arenosa) de áreas cultivadas com milho em aplicação de S-metolachlor, terbuthylazine, e mesotrione isolado e em uma mistura. A metodologia foi estabelecida de acordo com os microorganismos do solo: teste de transformação de carbono com uma solução de ^{14}C-glicose (D-[U-^{14}C] glucose) em frascos biométricos. Depois da adição de ^{14}C-glicose, a quantidade de ^{14}C no CO_2 acumulado da respiração microbiana foi medida várias vezes durante aos 28 dias de incubação. Para o solo sem alterações - controle (sem aplicação de herbicida), a atividade microbiana seguiu um comportamento similar ao solo tratado com herbicidas no total de $^{14}CO_2$ liberado e acumulado, variando de 23 a 27%. No geral, os valores da taxa constante de mineralização (k) para todos os tratamentos também foram semelhantes, com um valor médio de 0,0038% CO_2 d^{-1}, consequentemente os tempos de meia-vida da mineralização (MT50) foram de 173 a 198 d. A respiração microbiana em todos os tratamentos foi ligeiramente superior no argilo-arenoso em comparação com o solo franco-arenoso; embora as amostras de solo com aplicação de herbicidas (isolado e em uma mistura) não têm diminuído a respiração microbiana basal ou as taxas de mineralização da glicose. Para corroborar esses dados encontrados, são necessárias pesquisas adicionais com diferentes substratos orgânicos e em culturas com diferentes aplicações de herbicidas para comprovar a não interferência desses herbicidas na respiração microbiana no solo.

Palavras-chave: respiração microbiana, textura de solo, solo tropical.

Abstract

Microbial adaptation may occur in surface soils under agricultural uses exposed to herbicides. However, little is known about herbicide mixture effects in the soil, especially in tropical regions like Brazil. The aim of this study was to evaluate glucose mineralization in soils of contrasting textures (sandy clay and sandy loam) from areas cultivated with maize under application of S-metolachlor, terbuthylazine, and mesotrione alone and in a mixture. The methodology was established according to the soil microorganisms: carbon transformation test with ^{14}C-glucose solution (D-[U-^{14}C] glucose) in biometric flasks. After the addition of ^{14}C-glucose, the amount of ^{14}C in cumulative CO_2 of microbial respiration was measured several times during the 28-day incubation. For unamended soil – control (without herbicide), microbial activity followed a similar behavior to amended soil with herbicides in total $^{14}CO_2$

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[7] MENDES, K.F.; COLLEGARI, S.A.; PIMPINATO, R.F.; TORNISIELO, V.L. Glucose mineralization in soils of contrasting textures under application of S-metolachlor, terbuthylazine, and mesotrione alone and in a mixture. **Bragantia**, Campinas, 2017 (accepted manuscript).

released and accumulated, ranging from 23 to 27%. Overall, mineralization constant rate (k) values for all treatments were also similar, with an average value of 0.0038% CO_2 d^{-1}, consequently mineralization half-life times (MT50) were from 173 to 198 d. Microbial respiration for all treatments was slightly higher in the sandy clay compared with sandy loam soil; although soil samples with application of herbicides (alone and in a mixture) did not show decreased basal microbial respiration or mineralization rates of glucose. To corroborate these findings, additional research with different organic substrates and in cultures with different applications of herbicides are needed to prove the non interference of these herbicides on the microbial respiration in the soil.

Keywords: microbial respiration, soil texture, tropical soil.

6.1. Introduction

Microbial activity influences the dynamics of nutrients in the soil, promoting the decomposition of soil organic matter - SOM (mineralization) and solubilization of nutrients in the soil solid phase. These organisms also have other important functions such as the suppression of pathogens, the production of phytohormones and decomposition of pesticides, including herbicides; and multitrophic level microbial interactions drive eco- and agro-biotechnological processes such as bioremediation, wastewater treatment, plant growth promotion, and ecosystem functioning (BOTTOMLEY, 2005; SALEEM; MOE, 2014).

However, microbial adaptation may occur in surface soil under agricultural uses exposed to herbicides, interfering positively, providing for the metabolism of these products by the microorganisms or genetically modified or native microbes produce herbicide-degradaing enzymes that can mineralize different groups of herbicides and their metabolites with greater efficiency as a powerful technology for in situ remediation (REIS et al., 2008; HUSSAIN et al., 2009a), interfering negatively by intoxicating soil biota (non-adapted organisms) or have not effect (PEREIRA et al., 2008; MAHÍA et al., 2008; BLUME; REICHERT, 2015). Soils rich in SOM and with residual concentration of herbicides may increase the herbicide degradation rate, as shown for atrazine by Mirgain et al. (1993) in laboratory microcosm conditions, possibly because of microbial adaptation to repeated herbicide exposure.

S-metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]acetamide), terbuthylazine (6-chloro-*N*-(1,1-dimethylethyl)-*N*'-ethyl-1,3,5-triazine-2,4-diamine), and mesotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione) are widely used in pre- or early post-emergence to control weeds in maize. S-metolachlor is a member of the chloroacetanilide family and inhibits the very long chain fatty acid (VLCFA) formation, which interferes with normal cell development and inhibits both cell division and growth (TRENKAMP; MARTIN; TIETJEN, 2004). Terbuthylazine is a member of the triazine family and inhibits photosynthesis by inhibiting electron transfer at the reducing site of photosystem II in the chloroplasts (GOOD, 1961). Mesotrione is a weak acid, member of the triketone family of herbicides. Susceptible plants are controlled through inhibition of the 4-hydroxyphenyl-pyruvate dioxygenase (4-HPPD) enzyme, affecting carotenoid biosynthesis (MITCHELL et al., 2001). Given the high efficiency of these herbicides in weed control, a commercial mixture is recommended, e.g. Lumax® used in Italy (PINNA et al., 2014; OTTO et al., 2016), with label dose of 37.5, 212.5, and 187.5 g L^{-1} of mesotrione, S-metolachlor, and terbuthylazine, respectively.

A little is known about the impact of herbicide mixtures on soil (JOLY et al., 2015), especially in tropical regions like Brazil. Studies regarding the behavior of herbicides in the soil are usually carried out considering single molecules. However, Mendes et al. (2016) found that mesotrione, applied both alone and in a mixture with S-metolachlor + terbuthylazine, had no influence on its sorption or desorption, but Mendes et al. (2017) reported that mesotrione degradation rate was influenced by soil texture regardless if applied alone or in mixture. Concurrently, mesotrione sorption and biotransformation in the soil is relatively low and quick, respectively, indicating leaching potential, which can enter the groundwater in maize production fields. But little is known about the interference of these herbicides on the microbial respiration of soils, which can be measured by ^{14}C-labeled glucose mineralization in the amount of ^{14}C in CO_2 (TIAN et al., 2015).

The aim of this study was to evaluate ^{14}C-labeled glucose mineralization in tropical soils of contrasting textures from areas cultivated with maize under application of S-metolachlor, terbuthylazine, and mesotrione alone and in a mixture. Information on microbial respiration of these herbicides in the soils is crucial in assessing environmental impact and risk from the chemical applications.

6.2. Materials and Methods

6.2.1. Soil sampling and preparation

The carbon transformation experiments with the herbicides S-metolachlor, terbuthylazine, and mesotrione alone and in mixture were performed at the Ecotoxicology Laboratory of the Center of Nuclear Energy in Agriculture - CENA, University of São Paulo - USP, Piracicaba, SP, Brazil. The methodology was established according to the guidelines of the OECD – 217, Soil Microorganisms: Carbon Transformation Test (OECD, 2000).

Soil samples with contrasting textures were collected from the surface layer (0-10 cm depth), after pre-cleaning the residue or vegetation layer in two different locations from soil under maize cultivation in Piracicaba, SP, Brazil

(Alfisol – Paleudult, sandy clay, S 22°42’34”, W 47°37’18” and Ultisol - Typic Hapludalf, sandy loam, S 22°42’52”, W 47°37’10”). After drying, samples were sieved through a 2.0 mm mesh and stored at room temperature. The physical and chemical properties of the samples and classification are shown in Table 6.1.

Table 6.1 - Physicochemical properties of contrasting soil textures (0-10 cm of depth) studied in this experiment.

Soil[a]	pH	K	Ca^{2+}	Mg^{2+}	H + Al	BS	CEC
	(H_2O)				($mmol_c\ kg^{-1}$)		
NVef	6.4	11	51	26	41	88	129
PVAd	6.9	1	18	7	29	26	55

Soil[a]	P	V	OC	sand	clay	silt	texture class
	($mg\ kg^{-1}$)	(%)	(%)		(%)		
NVef	18	68	1.80	46.6	37.6	15.8	sandy clay
PVAd	15	47	0.52	81.6	15.1	3.3	sandy loam

[a] According to the Soil Taxonomy and Brazilian Soil Science Society (EMBRAPA, 2013). Nitossolo Vermelho eutroférrico – NVef (Alfisol – Paleudult) and Argissolo Vermelho-Amarelo distrófico – PVAd (Ultisol - Typic Hapludalf). pH = potential of hydrogen; K = potassium; Ca = calcium; Mg = magnesium; H + Al: potential acidity; BS = base saturation; CEC = cation exchange capacity; P = phosphorus; V = base saturation levels; OC = organic carbon. **Source:** Soil Science Department - ESALQ/USP, Piracicaba, SP, Brazil.

6.2.2. Incubation and microbial respiration

Soil samples (300 g, dry weight) were placed into 3,000 mL jars. The moisture content was adjusted to 50% of the water holding capacity (WHC), and the soil was then pre-incubated at $20\underline{+}2^oC$ for one week. The soil then reached a final soil moisture content of 75% WHC by mixing water deionized. In total, a 2x5x5 factorial experiment was established corresponding to two soils (sandy clay and sandy loam), five herbicide types (control – without herbicide, S-metolachlor + terbuthylazine + mesotrione, S-metolachlor, terbuthylazine, and mesotrione), and five incubation times (0, 7, 14, 21 and 28 days) prior to glucose addition.

After application of herbicides doses in the soil samples, they were mixed carefully with a spatula assuring complete homogenisation of the samples. After application and homogenization of the soil sample a 10 g aliquot was taken from each initial sample (300 g) and transferred to a biometric flask (250 mL). This procedure was performed in triplicate.

An aliquot of 1 mL of standard analytical ^{14}C-glucose solution (D-[U-^{14}C] glucose) with specific activity of 11 GBq $mmoL^{-1}$ and total activity of 37 MBq mC^{-1}

were added to soil samples of each biometric flask at 0, 7, 14, 21 and 28 days after herbicide application.

After application of ^{14}C-glucose solution, each biometric flask was sealed with a rubber stopper attached to a filter "lime soda"; containing stopper between the filter and the flask. The entry of atmospheric CO_2 into the flask was blocked by filter "lime soda" ensuring that $^{14}CO_2$ was collected from the microbial respiration only. An aliquot of 10 mL of a sodium hydroxide (NaOH) solution 0.2 mol L^{-1} were added in all lateral tubes of each biometric flask.

Aliquots (1 mL) of radiolabeled solutions of NaOH were collected 6 h after application of ^{14}C-glucose solution and transferred in duplicate to separate vials containing 10 mL of the scintillation solution insta-gel plus, and the initial concentration of ^{14}C-glucose after 15 min was determined by liquid scintillation counting with a Tri-Carb 2910 TR LSA counter (PerkinElmer). The remaining solution of NaOH was removed from the lateral tube and then it was again filled with 10 mL of a NaOH new solution (non-radiolabeled).

6.2.3. Chemical products

The stock solutions (1,200 µL) were prepared using non-radiolabeled analytical standards of mesotrione (150 g ha^{-1}), S-metolachlor (1250 g ha^{-1}) and terbuthylazine (750 g ha^{-1}) alone and mixture with purities of 99.9, 98.2 and 98.8%, respectively (Sigma Aldrich, Saint Louis, MO, USA). Herbicide doses were calculated according to the collection depth of 0.1 m, soil density 1,200 kg m^{-3} and 300 g soil mass on a dry basis. Non-radiolabeled standards were carefully mixed in acetone to reach the final volume of stock solution.

6.2.4. Mineralization rates

A kinetic assessment of mineralization rates was conducted to compare different treatments. First-order reaction models were fitted to observe cumulative evolved CO_2 for each herbicide (control, S-metolachlor + terbuthylazine + mesotrione, S-metolachlor, terbuthylazine, and mesotrione), using linear and nonlinear regression analyses (BLUME; REICHERT, 2015). The selection of model order was based on goodness-of-fit of the model to observed data, measured by the coefficient of determination (R^2).

6.2.5. Statistical data processing

An analysis of variance (ANOVA) was used to detect differences in herbicide treatments within each soil and incubation time. When significant, means were compared by Tukey's test ($p<0.05$), whereas mineralization kinetic and decomposition parameters were estimated by the Sigma Plot® (Version 10.0 for Windows, Systat Software Inc., Point Richmond, CA).

6.3. Results and Discussion

6.3.1. Microbial respiration

The triple interaction between soil type, herbicide type, and incubation time was statistically significant. Cumulative $^{14}CO_2$ (% of added ^{14}C-glucose) of microbial respiration for all 5 herbicide types (control – without herbicide, S-metolachlor + terbuthylazine + mesotrione, S-metolachlor, terbuthylazine, and mesotrione) in the different incubations was higher statistically ($p<0.05$) in the sandy clay compared with sandy loam soil; however it exhibited a similar behavior with steady growth exponential over time (Figure 6.1). This fact can be attributed to higher OC (1.80%) and clay (37.6%) content in the sandy clay in relation to the sandy loam soil (0.52% and 15.1%, respectively) (Table 6.1). In the same conditions of this experiment, Mendes et al. (2017) also found that mineralized ^{14}C-mesotrione accumulation (alone and in a mixture with S-metolachlor + terbuthylazine), represented by $^{14}CO_2$ accumulation, was lower in the sandy loam (65–70%) than in the sandy clay soil (85–83%). The same authors described that difference in mesotrione present in the soil solution over time between the two soil types may be attributed to the difference in the OC content and pH of the soils, which are directly related to the difference in microbial communities' type and amount. However, assessing microbial activity of the soils would be a valuable data.

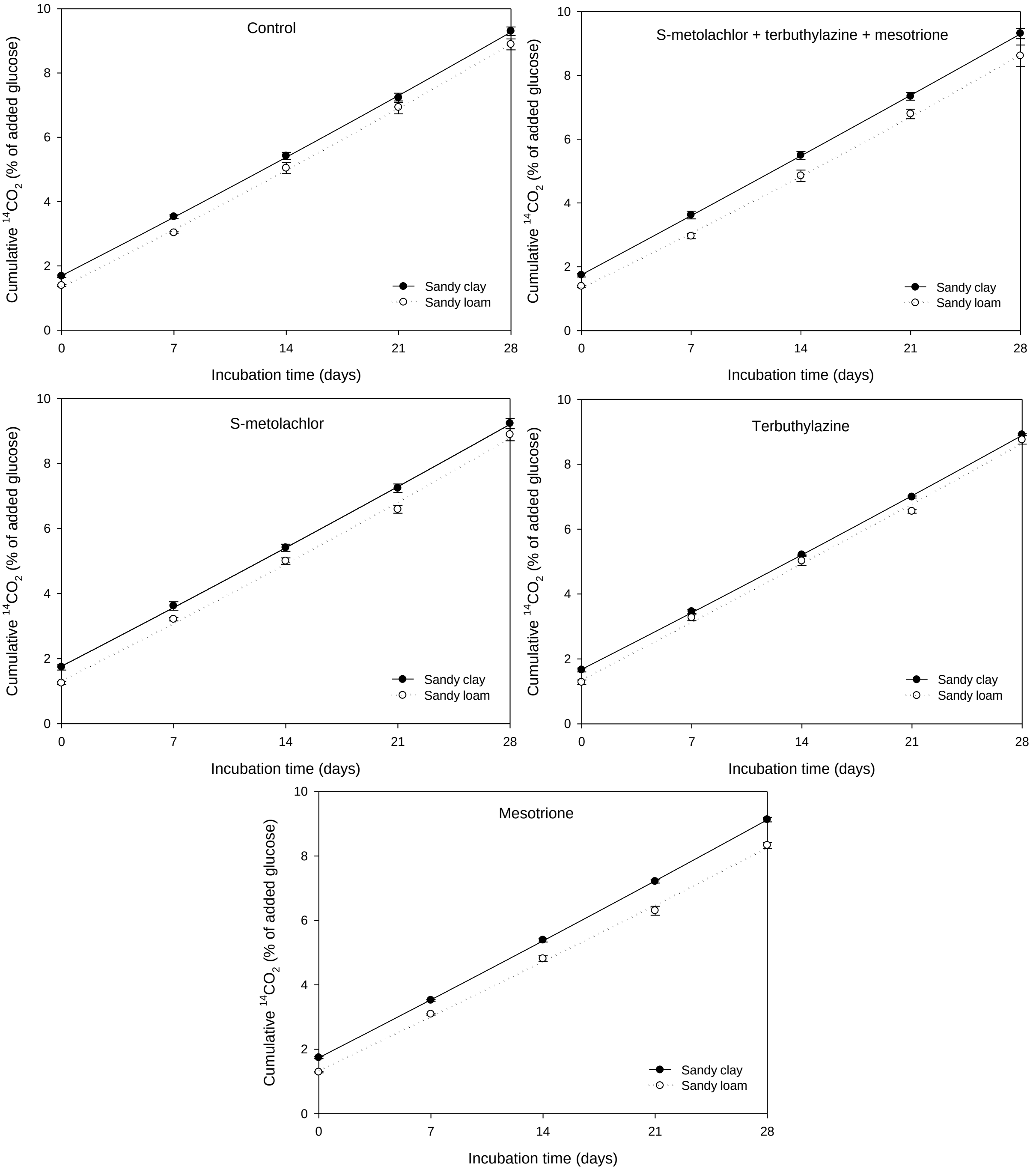
Soil microorganisms degrade natural and synthetic organic compounds whereas their degradation products may either accelerate or decrease microbial activities (SCHMIDT et al., 2011; HUSSAIN et al., 2009b). As a result, environmental change, e.g. herbicides used for weed control can influence soil carbon cycling through changes in both metabolic activity and community structure. Tejada (2009) reported that the application of a glyphosate + diflufenican mixture to soil increased the inhibition of the soil microbial biomass-C and soil enzymatic activities compared to the action of both herbicides applied individually. However, the persistence period of the herbicides depended on the soil texture, and influences its toxic effect, corroborating with data found in the present study.

Initially at 0 d of incubation of both soils, 1.2–1.7% of the added glucose was mineralized, and up to 8-9% of the glucose was mineralized within 28 days (Figure 6.1). Tian et al. (2015) reported that high glucose addition (204 µg Cg^{-1} soil) increased the percentage of glucose-C mineralized to CO_2, but it decreased the proportion of the added glucose incorporated into microbial biomass, compared to the low glucose level (20.4 µg Cg^{-1} soil). Thus, future studies are required to integrate the soil microbial community structure with the different levels of glucose additions.

Blume and Reichert (2015) reported that adding substrates (glucose and ground banana leaves) to soil increased microbial respiration for all sites [5 banana plantations with diverse pesticide management (herbicide, nematicide, and fungicide), plantation age (5 yr and 20 yr)], and microhabitats (bare area, litter pile, and nematicide ring). With the addition of glucose, a readily degradable substrate, no differences were observed for plantation age or pesticide use rate. Independent of plantation history, soil microorganisms were capable of responding to a simple organic carbon source. In addition, the same authors described that by adding a greater amount of carbon from banana leaves (0.25 g compared with 0.05 g glucose), nitrogen immobilization may reduce its availability to microorganisms and plants.

Overall, cumulative $^{14}CO_2$ of microbial respiration for treatments: S-metolachlor + terbuthylazine + mesotrione, S-metolachlor, terbuthylazine, and mesotrione were very similar in the sandy clay and sandy loam soil, showing that addition of three herbicides in mixture did not affect the microbial respiration compared to treatment with each herbicide alone or to control, without herbicides (Figure 6.1). These results are comparable with previous studies, which reported that soil samples from high pesticide input (application of herbicides, nematicides, and fungicides) did not have decreased basal microbial respiration or mineralization rates, because some pesticides’ residues could be carbon or energy source to microorganisms and are degraded and assimilated by these microorganisms (HUSSAIN et al., 2009b; BLUME; REICHERT, 2015).

Figure 6.1 - Cumulative $^{14}CO_2$ (% of added ^{14}C-glucose) of microbial respiration for 5 herbicide treatments (control – without herbicide, S-metolachlor + terbuthylazine + mesotrione, S-metolachlor, terbuthylazine, and mesotrione) and different incubation periods in sand clay and sandy loam soil. Error bars represent standard error of the means (*n* = 3).



Joly et al. (2015) also reported that although the pollution pressure was maintained throughout the experiment (by herbicides: S-metolachlor, mesotrione, and nicosulfuron; adjuvants, and/or degradation products), the Limagne soil microbial communities appeared to be quite resistant to the different treatments from a global point of view; and that the bacterial and fungal diversity estimated by fingerprinting analyses remained unchanged, such as microbial biomass estimated by the microbial carbon measurements in the presence of metolachlor (WHITE et al., 2010). This absence of effects, already shown by other authors concerning herbicide mixtures (JOLY et al., 2015), could be explained by a real absence of effect or by the hypothesis of functional redundancy proposed by Wardle and Parkinson (1990), whereby microbial communities under the effect of herbicides were presumably in a considered state of flux, with susceptible microbes being killed and others, thereby, having a readily available source of carbon, explaining a balance in the microbial parameters.

6.3.2. Mineralization rates

The response of microbial communities to applications of S-metolachlor, terbuthylazine, and mesotrione alone and in a mixture and the mineralization rates of glucose are describable by reaction kinetics. We calculated decay constants (k) for site only (Table 6.2) without correction for microbial biosynthesis; thus, the constants reflect the net mineralization rates as described by Blume and Reichert (2015).

For unamended soil – control (without herbicide), microbial activity followed a similar behavior to amended soil with herbicides in total $^{14}CO_2$ released and accumulated, ranging from 23 to 27% (Table 6.2). In the sandy clay, a lower amount of CO_2 was observed in the soil-applied terbuthylazine (~26%), and in the sandy loam, a higher amount of CO_2 was observed in the soil-applied herbicides mixture (S-metolachlor + terbuthylazine + mesotrione) with ~27% and a lower amount was observed in the soil-applied mesotrione (~23%). Therefore no microbial toxic effects were observed from the chemical mixtures with respect to reductions in the microbial community, nor in reduced glucose mineralization. There was little difference between the types of soil, as previously indicated, and this difference is probably not having an environmental impact on the microbial community. S-metolachlor, terbuthylazine, and mesotrione applied alone at the recommended field rates exert only few or not consistent minor effects on soil microbial communities and its influence depended on the rate of application and duration of activity (SALMINEN; ERIKSSON; HAIMI, 1996; JOLY et al., 2012; RADIVOJEVIC et al., 2013). Overall, k values for all treatments were also similar, with an average value of 0.0038% CO_2 d^{-1}, consequently mineralization half-life times (MT50) were from 173 to 198 d.

Table 6.2 - $^{14}CO_2$ released and accumulated at the 28th day of incubation (%) and parameters of the first order kinetics (mineralization constant rate - k, mineralization half-life - MT50, and coefficient of determination - R^2) of the applied ^{14}C-glucose in sand clay and sandy loam soil with S-metolachlor + terbuthylazine + mesotrione, S-metolachlor, terbuthylazine, mesotrione, and without herbicide (control).

Herbicide	Texture soil	Parameter			
		$^{14}CO_2$ (%)	k (d^{-1})	MT50 (d)	R^2
Control	sandy clay	27.00 Aa	0.0039	177.73	0.99
	sandy loam	24.63 Bb	0.0037	187.33	0.99
S-metolachlor+terbuthylazine +mesotrione	sandy clay	27.42 Aa	0.0040	173.28	0.99
	sandy loam	25.30 Ba	0.0038	182.40	0.99
S-metolachlor	sandy clay	27.07 Aa	0.0039	177.73	0.99
	sandy loam	24.26 Bb	0.0037	187.33	0.99
Terbuthylazine	sandy clay	26.15 Ab	0.0038	182.40	0.99
	sandy loam	24.29 Bb	0.0037	187.33	0.99
Mesotrione	sandy clay	26.91 Aa	0.0038	182.40	0.99
	sandy loam	23.34 Bc	0.0035	198.04	0.99

Means followed by the same capital letter for each herbicide and small caps with respect to soil type do not differ by Tukey's test (p<0.05). DMS (soil) = 0.0861, DMS (herbicide) = 0.1206, and and CV(%) = 2.32.

Blume and Reichert (2015) reported that decreased k for long-term cultivation independently of pesticide input clearly shows an age effect on mineralization rate for readily decomposable organic material. It is possible that high SOM maintains an active microbial population in the field, which is stimulated by readily degradable carbon sources. Thus, continuous input of fresh organic materials is required to maintain adequate microbial activity for sustainable crop production in the long run. Therefore, other factors like soil properties, nature, and concentration of herbicide used, its activity and production of metabolites during metabolism in soil also contribute to determining the effect of herbicides on soil biological activities (HUSSAIN et al., 2009b).

Agricultural soils receiving herbicide mixtures differ in their mineral and organic composition: essential factors in the herbicide sorption, bioavailability and degradation processes, which prevent us to conclude at a larger scale on the safety of the use of this three herbicide mixture (JOLY et al., 2015). Knowing that herbicide mixtures is the current trend in agricultural practices, it is essential that we investigate more precisely their impact with more sensitive methodologies and approaches, and focus on specific microbial communities which ensure key functional steps in biogeochemical cycles (JOLY et al., 2012). Generally, understanding the mechanisms underlying molecular responses in microorganisms in response to herbicides application could be helpful in elucidating the risk assessment of herbicides contaminations and its consequent adverse impacts on soil microbial diversity, enzymatic activities, and biochemical reactions (HUSSAIN et al., 2009b).

6.4. Conclusions

According the above results, we conclude that microbial respiration for all treatments was slightly higher in the sandy clay compared with the sandy loam soil. Soils of contrasting textures from areas cultivated with maize under application of S-metolachlor, terbuthylazine, and mesotrione alone and in a mixture did not have decreased basal microbial respiration or mineralization rate of glucose. To corroborate these findings, additional research with different organic substrates and in cultures with different applications of herbicides are needed to prove the non interference of these herbicides on the microbial respiration in the soil.

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7. GENERAL CONCLUSIONS

Mesotrione, applied both alone and in a mixture with S-metolachlor + terbuthylazine, had no influence on its sorption, desorption or leaching. Both application modes exhibited similar retentions in the soil. Concurrently, mesotrione sorption in the soil is relatively low, indicating relatively high leaching potential, and thus can present potential groundwater contamination risks and therefore surface water in maize production fields.

Among the physicochemical properties of soils cultivated with maize, the pH and the CM content presented linear correlations with mesotrione sorption and leaching, allowing an understanding of key parameters responsible for the behavior of this herbicide in arable areas of Brazil. Our results should aid the predictability of mesotrione sorption in soils with similar properties. Thus, the use of mesotrione without the prior knowledge of the physical and chemical properties of the topical soil, can result in inefficient weed control and high product leaching risks.

Study on the quantification of the fate of herbicides alone and in mixture in the soil is of outmost importance, considering the number of herbicides mixture that are currently used in different soil types and climatic conditions. Further studies of the fate of herbicides mixture in soil may provide insight into the mechanism of degradation of other herbicides and may suggest ways in which mixture may affect to microbial activity in the soil. Mesotrione biotransformation was relatively quick, indicating this herbicide has low persistence, and consequently, low residual effect on crops and weeds when present in similar soils to this present study.

Microbial respiration in soil with mesotrione, S-metolachlor, and terbuthylazine was slightly higher in the sandy clay compared with sandy loam soil, but these herbicides applied alone or in a mixture at recommended doses did not have decreased basal microbial respiration or mineralization rate of glucose. However it is evident the effect absence (non-toxic) of these herbicides on microbial activity in the soil, but herbicides effects on the microbiological and enzymatic properties of soil need to be more studied.

APPENDIX

Alachlor availability in biochar amended soils and uniform tillage systems[8]

ABSTRACT: Agricultural and soil management practices have been reported to affect alachlor sorption-desorption and degradation rates. The objectives of this study were to: (a) assess differences in sorption due to tillage treatments (chisel plow and ridge tillage) on sorption of alachlor from three Midwestern U.S. states, a silt loam (Minnesota and Pennsylvania) and a silty clay loam (Illinois); (b) determine the effect of amending soils with biochars derived from soybean residues, sugarcane bagasse, and wood chips (pine) derived biochars, or same raw feedstocks, on the sorption-desorption and mineralization of alachlor. Soil was amended at 10% (w/w), and sorption-desorption studies were performed in duplicate using the batch equilibration method. Soils were treated with ^{14}C-alachlor, and incubated for 30 days to determine mineralization. Surprisingly, tillage management did not affect alachlor sorption to soil when comparing tillage system and row position across all three sites, despite the fact that the tillage operations were imposed for 4 years (P>0.05). While sorption coefficient (K_d) values for alachlor were relatively low in the unamended three soils (K_d = 1.76, 1.73, and 1.15 L kg^{-1} for IL, MN, and PA soils, respectively, sorption of alachlor increased from 4 to 33-times in a biochar-amended soils as compared to the unamended soil. Mineralization of alachlor was slower in biochar or raw feedstocks amended soils. Therefore, the composition of the biochar in the soils amended can play an important role in the sorption-desorption and mineralization of alachlor.

Keywords: black carbon, nonionized herbicide, management practices, soil behavior.

Highlights

- Ridge tillage and chisel plough by position (ridge and furrow) didn’t affect the alachlor sorption.
- In biochar-amended soils have implications for alachlor sorption-desorption efficacy.

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[8] MENDES, K.F.; SPOKAS, K.A.; HALL, K.E.; KOSKINEN, W.C.; TORNISIELO, V.L. Alachlor availability in biochar amended soils and uniform tillage systems. **Science of the Total Environment,** Amsterdam, 2017 (submitted manuscript).

- Mineralization of alachlor was affected by biochar amended soils.

Abbreviations: k = first-order reaction rate constant; MT_{50} = mineralization time half-life; OC = organic carbon; RT = ridge tillage; CP = chisel plough.

1. Introduction

Alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide] is a soil-applied chloroacetamide herbicide widely used as a pre-emergent, early post-emergent, or pre-plant incorporated herbicide for control of grasses or broadleaf species, under a variety of tillage and crop residue management systems (Locke et al., 1996). Typically sorption of alachlor has been correlated with organic carbon content, with higher carbon containing materials resulting in reduced leaching due to increased sorbed amounts (Guo et al., 1993).

Conservation tillage practices that leave plant residues at the soil surface have become a significant part of soil conservation plans, because reduce soil erosion, conserve soil moisture, and may also offer other benefits such as improved soil tilth, buildup of soil organic C, and altered microbial populations (Reddy et al., 1995). Changing tillage practices can also change herbicide performance, particularly for soil active herbicides. Herbicide performance can be influenced by the amount of soil disturbance, the degree of incorporation of the herbicide, the position of weed seeds in the soil and the amount of plant residue present. There has been a considerable amount of research on the interactions of tillage and herbicide performance, mostly conducted overseas (Chauhan et al., 2006). Although, implementation of conservation tillage induces an increase in organic matter content at the soil surface and its gradual decrease with depth. This, in turn, leads to an increase in herbicide retention in the topsoil layer (Alletto et al., 2010).

The affinity of a herbicide for soil organic C has practical implications in terms of herbicide distribution. Herbicide binding can be weak or strong depending on degree of decomposition of plant residue. Increased herbicide adsorption to soil organic C decreases the availability of herbicide to plants and microorganisms. Accumulation of well-decomposed plant residues may increase adsorption and prolong herbicide residence time at the soil surface. Increased adsorption can reduce herbicide leaching but may result in inadequate weed control because less herbicide is available for target weed uptake. Knowledge of herbicide fate, transformation, and transport can be used to adjust application rates to obtain effective short-term weed control (from herbicide in solution) and long-term weed control (from sorbed herbicide that would desorb with time). Although no-tillage systems are increasingly becoming accepted, more information is needed on the effects of these systems on herbicide fate in soft (Reddy et al., 1995). Zonal tillage (e.g. ridge tillage, RT) separates management of row and inter-row positions, while non-zonal tillage (e.g. chisel plough, CP) applies management uniformly across a field (Williams et al., 2016).

One area in the renewable energy renaissance attracting significant attention is the use of biochar produced from the pyrolysis of vegetative biomass (Spokas and Reicosky, 2009). Biomass sources such as agricultural residues or forestry wastes (e.g. wood chips, soybean residues, and sugarcane bagasse) are excellent precursors for the production of bio-oil, biochar and biogas energy products.

Given the similarities in some of properties of biochars and their similar effects on the fate processes of herbicides, the existing body of the literature on biochar is bioaccessibility, bioavailability, efficacy, and toxicological impact are directly linked to the desorption behavior, as the compound needs to be released back into soil solution for efficacy (Kookana et al., 2011). Studies have demonstrated implications for herbicide sorption and desorption efficacy in biochar amended soils (Spokas et al., 2009, Zheng et al., 2010, Martin et al., 2012). As a consequence, weed control in biochar-amended soils may prove more difficult as pre-emergent herbicides may be less effective and require additional application amounts (Kookana et al., 2011).

Sorption and bioavailability of alachlor is correlated with organic matter, clay content, surface area, and CEC (Koskinen et al., 2003; Peter and Weber, 1985). As organic matter and clay content increase, sorption increases and bioavailability decreases (Peter and Weber, 1985). Weber and Peter (1982) proposed a mechanism of sorption in which carbonyl oxygen atoms of alachlor were coordinated with the calcium atoms on the clay surface, perhaps on the interlayer surfaces of the clay.

Agricultural and soil management practices have also been reported to affect alachlor sorption-desorption rates (Guo et al., 1993, Locke et al., 1996, Dorado et al., 2005, Dal Bosco et al., 2013) and mineralization (Guo et al., 1991, Liu et al., 2002). The objectives of this study were to: (a) determine sorption of alachlor applied to soils from three Midwestern U.S. states [silt loam (Minnesota and Pennsylvania) and a silty clay loam (Illinois)]. The soils were from fields under ridge tillage (RT) (ridge and furrow) and chisel plough (CP); (b) determine the effect of amending soils with biochars derived from soybean residues, sugarcane bagasse, and wood chips (pine) derived biochars, or same raw feedstocks, on the sorption-desorption and mineralization of alachlor.

2. Materials and Methods

2.1. Soil characterization and experimental design

Three Midwestern U.S. soils were selected for this study: silt loams from Minnesota (MN) and Pennsylvania (PA), and a silty clay loam from Illinois (IL). At each location the experiment was established as a randomized complete block design with four replicates (blocks). Within each block there were four plots: two ridge tillage (RT) and two chisel plough (CP). For both CP and RT plots, one plot was under corn (*Zea mays* L.) and one was under soybean (*Glycine max* (L.) Merr.); crops were rotated between corn and soybeans annually.

These field plots were established in 2011 and planted with maize. Prior to 2011, IL and MN were managed under maize-soybean rotations using conventional, uniform tillage, while PA was under sorghum (*Sorghum bicolor* L. Moench). Beginning in 2012, the tillage treatments were established and managed under the annual maize-soybean rotation described above. Thus, the RT plots are in an early stage of transition from conventional to reduced tillage. Permanent ridges were formed in RT, and in both rotations maize and soybean were planted at the center of ridge tops. Crop residues were concentrated onto the soil surface of furrows during planting. RT ridges were re-ridged [furrow surface soil scraped back onto ridge (Hatfield et al., 1998)] shortly after the maize six leaf stage (V6). In CP, maize and soybean were planted into level, cultivated soil, i.e. no ridges, and crop residues were ripped and incorporated into the soil during cultivation. In both tillage systems, weeds were sprayed with glyphosate three weeks prior to planting. Row/ridge widths varied by site, being 30 cm at IL, 25 cm at MN, and 30 cm at PA. Management varied at each site in accordance with local best management practices, as outlined in Williams et al. (2016).

From each plot, soil samples were collected from the upper 5 cm of ridges and furrows in the RT soils and upper 5 cm of CP plots, air-dried, and passed through a 2 mm sieve. Selected soil properties are listed in Table 1.

Analyses of soil texture, OC content, and total nitrogen (N) were performed by the University of Minnesota Soil Testing Laboratory (St. Paul, MN). Soil texture was determined using the hydrometer method. Soil OC content was determined by dry combustion at 900°C and measurement of CO_2 evolution using a C/N Analyzer (VarioMAX; Elementa Americas, Inc., Mt. Laurel, NJ).

2.2. Alachlor herbicide

Solutions were prepared with $CaCl_2$ (0.01 M) in the concentration alachlor (1 mg L^{-1}). All solutions were spiked with the corresponding ^{14}C radiolabeled herbicide to give solution radioactivity level of 400 Bq mL^{-1}. Radiolabeled (UL-ring-^{14}C) alachlor was obtained from Pathfinder Laboratories (St. Louis, MO). Specific activity was from 500 kBq $mmol^{-1}$ and radiochemical purity was >97%.

Table 1. Soil profiles (0-10 cm of depth) for the three sites.

Site	Soil taxonomy[a]	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})	SOM (g kg^{-1})	CEC (meq 100 g^{-1})	pH	Bray P (mg kg^{-1})	K (mg kg^{-1})	Ca (g kg^{-1})	Mg (mg kg^{-1})
MN	Fine-silty, over sandy, mixed, mesic Typic Hapludoll	280	560	160	42.5	14.5	6.7	40.1	169.2	2.2	445.3
IL	Fine-silty, mixed, superactive, mesic Typic Endoaquoll	170	560	270	47.9	16.6	6.0	25.1	187.9	2.5	444.4
PA	Fine, mixed, semiactive, mesic Typic Hapludalf	100	650	250	33.8	8.2	6.2	51.9	118.5	1.5	109.3

[a] According to USDA.

SOM: soil organic matter, CEC: cation exchange capacity, pH: potential of hydrogen, Bray P: phosphorus Bray extraction, K: potassium, Ca: calcium, Mg: magnesium.

2.3. Biochar amendment

Three biochars were prepared from soybean residues, sugarcane bagasse, and wood chips (pine) by heating for 2 hours at 500, 350, and 500°C, respectively. Biochar properties are listed in Table 2. The biochar was added to soil at a 10% (w/w) ratio. Despite being an impractical field application rate, this rate was used to elucidate the impact of biochar and the un-charred biomass on sorption behavior.

Table 2. Physico-chemical properties of the biochars.

Feedstocks	Production T (°C)	C (%)	N (%)	O (%)	S (%)	Ash (%)
Soybean Residues	500	48.0	1.26	11.07	0.03	37.5
Sugarcane Bagasse	350	75.2	0.66	15.76	0.06	3.76
Wood Chips (Pine)	500	87.2	0.43	6.44	0.01	2.37

2.4. Sorption-desorption experiment and model

Sorption experiments were performed in duplicate using the batch equilibration method, according Organization for Economic Co-operation and Development (OECD, 2000). For sorption experiments, a completely randomized batch experiment of a 2 x 7 x 2 factorial design, with the factors of tillage (2 types), 7 different amendments [1 control and 3 feedstocks (charred and uncharred)] and 2 replicates was established. The ratio of soil to solution for the herbicide was selected to achieve measurable sorption of the original chemical. In 50 mL glass centrifuge tubes with Teflon-lined caps, 20 mL of alachlor solution (1 µg/L; 10,000 dpm/mL) was added to 5 g of the respective treatment. The tubes were shaken horizontally for 24 hr in oscillating shaker in a dark room at 20 ± 2 °C. The 24 hr shaking time was sufficient for equilibration according to preliminary kinetics studies (data not shown). After equilibration, samples were centrifuged for 15 min at 1280x *g* and the supernatants were collected. One mL aliquots of the supernatant solutions were mixed with 5 mL scintillation cocktail [EcoLite(+)™, MP Biomedicals, LLC, Solon, OH] and analyzed for ^{14}C by liquid scintillation counting (LSC) using a Packard 1500 Tri-Carb counter (Packard Instruments, Downers Grover, Il).

Desorption experiments were performed immediately after sorption under the same conditions. An additional 9 mL of supernatant liquid was removed and replaced with 10 mL of 0.01 mol L^{-1} $CaCl_2$ solution. This volume (10 mL) was equal to that removed from the sorption experiment tubes, representing a reduction of 50% of the initial starting concentration. The tubes were shaken again for 24 h. After re-equilibration, the tubes were centrifuged and 1 mL aliquots of the supernatant were pipetted to scintillation vials containing 5 mL of scintillation cocktail and analyzed by LSC as described above. The desorbed amount was calculated as the difference between the radioactivity sorbed in the soil and in the remaining supernatant.

Percentage of alachlor adsorbed on the unamended or amended soil was calculated as: % sorption = $[(C_i - C_e)/C_i] \times 100$. Sorption coefficient ($K_d$, L kg^{-1}) was also calculated with the equation: $K_d = C_s/C_e$, where C_s is the amount of alachlor sorbed on the unamended or amended soil: $C_s = (C_i - C_e) \times V/M$, C_i being the herbicide initial liquid concentration, C_e being the equilibrium liquid concentration, V being the volume of herbicide solution added, and M is the mass of soil (Cabrera et al., 2014). The sorption coefficient normalized to the OC content of the soil (K_{oc}, L kg^{-1}) were calculated by the following: $K_{oc} = (K_d/(\%OC)) \times 100$. The desorption K_d value for the desorption was also calculated for comparison to the sorption K_d.

2.5. Mineralization experiment and model

The experiments were conducted according the methods established by the Organization for Economic Co-Operation and Development (OECD, 2002), in a completely randomized 3 x 2 factorial design with 2 replicates. The two factors were soils types amended with biochar (3 types: soybean residues, sugarcane bagasse, and wood chips (pine), or raw feedstocks). Each experimental unit consisted of a 250 mL biometer culture flask (Fisher; Part #C-4443-250) equipped with a side tube, which was contained 10 mL of 0.2 M sodium hydroxide (NaOH) as the carbon dioxide (CO_2) trap.

^{14}C-alachlor solutions were added dropwise using a microliter syringe to 5 g of soil amended with 0.5 g biochar or raw feedstock. The final amount of chemical added to soil samples equaled 1 mg kg^{-1} soil, a value close to the normal soil application rate of alachlor assuming an equivalent rate of 2.8 kg ha^{-1} and uniform distribution in the surface soil (assuming soil bulk density = 1200 kg m^{-3}, incorporation depth = 0.10 m). The moisture of soil samples was standardized to 20% gravimetric.

Soil samples were incubated at 28 ± 1 °C in the dark for up to 30 days. Vials with NaOH were replaced weekly (7, 14, 21 and 30 days) and flasks were aerated and moisture content was adjusted twice a week, if needed. The NaOH solution was completely removed at each sampling time. To determine the amount of evolved $^{14}CO_2$, a 1-mL aliquot of NaOH solution was added to 5 mL of scintillation cocktail [EcoLite(+)™, ICN Biomedicals, Costa Mesa, CA] and samples were left in the dark for 24 h. The concentration of ^{14}C in solution was determined by liquid scintillation counting (LSC) for 5 min in a liquid scintillation analyzer (Packard 1500 - Packard Instruments, Downer Grove, IL.).

Data of $^{14}CO_2$ produced and ^{14}C-alachlor were fit to a first order kinetic model: $C_t = C_0\ e^{-kt}$, where C_t is alachlor concentration at time t (%); C_0 is alachlor concentration at time zero; k is a mineralization rate constant (d^{-1}); and t is the incubation time (days). Mineralization half-life time (MT_{50}), defined as the time required for 50% of the applied herbicide to be mineralized to CO_2, were calculated by the following (Picton and Farenhorst, 2004): $MT_{50} = (\ln 2) / k$.

2.6. Statistical analysis

Alachlor sorption-desorption coefficients (K_d and K_{oc}) and mineralization (CO_2 production and MT_{50}) data were subjected to analysis of variance (ANOVA) and when significant differences between the treatments, averages were compared by Dunnett's honest significant difference (HSD) test ($p<0.05$). Figures were plotted using Sigma Plot® (Version 10.0 for Windows, Systat Software Inc., Point Richmond, CA).

3. Results and Discussion

3.1. Alachlor sorption in tillage systems

Despite the fact that the tillage management had a 4-year history at these sites, there was no significant difference in the sorption of alachlor due to the tillage managements. There was no statistically significant difference between the tillage type (RT and CP) or the row position (ridge and furrow) for alachlor sorption from the three sites (MN, IL, and PA) ($p > 0.05$; Tukey's test) (Fig. 1). The average sorption coefficients (K_d) were 1.76, 1.73, and 1.15 L kg^{-1} for IL, MN, and PA soils, respectively, with % sorption ranging from 20.0 ± 1.1 to 35.8 ± 0.4 in the three soils. This sorption percentage was correlated with the differences in the SOM (Table 1).

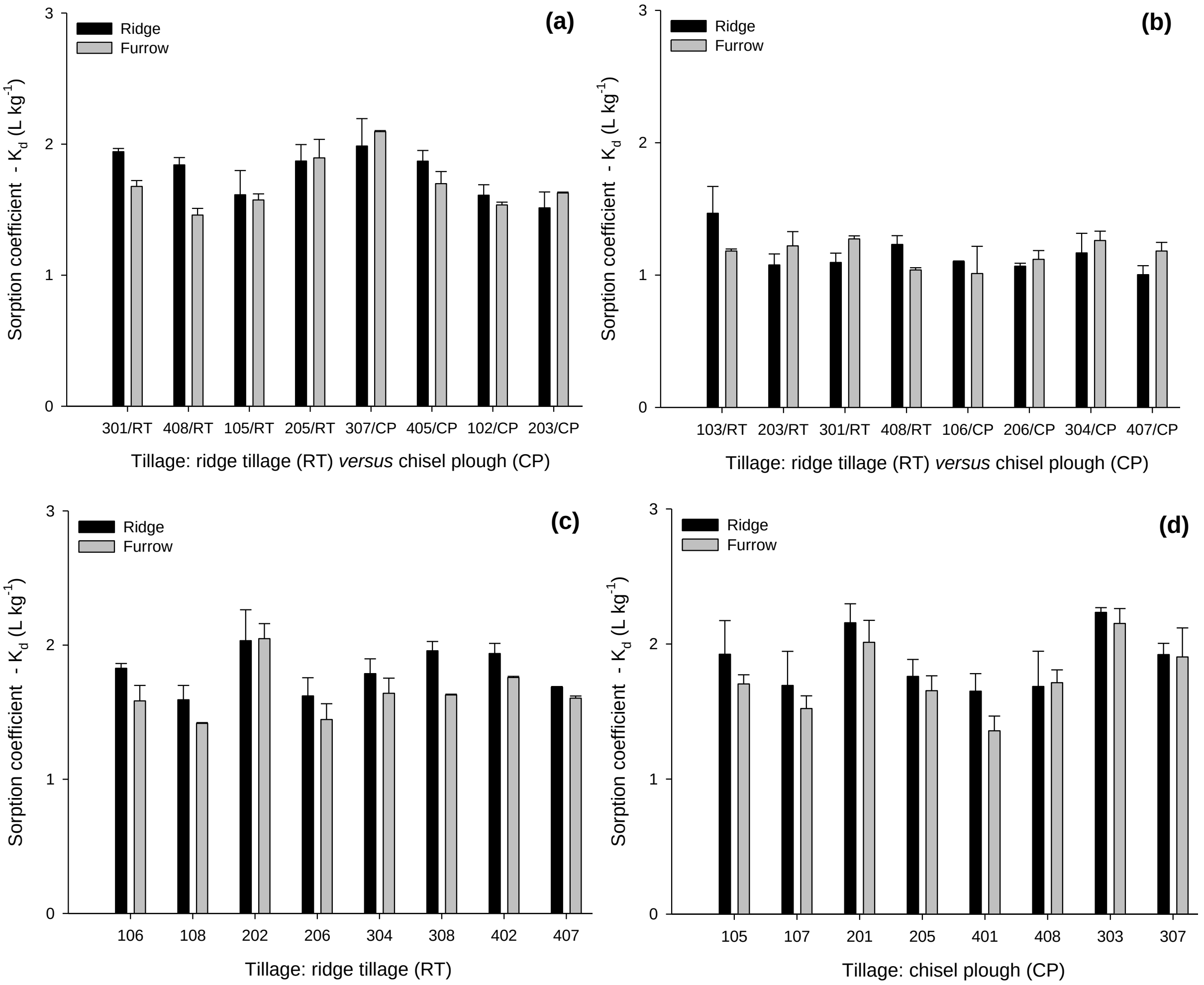

Fig. 1. Sorption coefficient - K_d (L kg^{-1}) of alachlor applied in soils from three states Americans [Minnesota, silt loam (a); Pennsylvania, silt loam (b); and Illinois, silty clay loam (c and d)] of the ridge tillage (RT) and chisel plough (CP) by position (ridge and furrow). The vertical bars associated with each column represent the standard deviation (±SD) of each mean value (*n* = 2).

Locke (1992) observed that no-tillage increased sorption compared to conventional tillage. However, in that study there was a larger difference in the organic carbon of the soils (1.02 and 1.67%), which could be responsible for that observation. Other studies have observed the reverse where no-till soil columns leached a higher amount of alachlor than conventional tillage (Clay et al., 1991) or no effect (Hansen et al., 2001).

Despite strong changes in soil physical properties under conservation tillage, herbicide transfer is more influenced by initial soil conditions and climatic conditions than by tillage, and conservation tillage systems such as no-tillage improve macropore connectivity, which in turn increases herbicide leaching (Alletto et al., 2010).

3.2. *Alachlor sorption-desorption in biochar amended soils*

As expected, sorption of alachlor increased in soil after the addition of biochar (Table 3), presumably as a result of the increase in carbon in the soil, as suggested by Spokas et al. (2009) who showed greater sorption of acetochlor, a related acetanilide herbicide, on biochar amended soil as compared to unamended soil. It should be noted sorption depends on the herbicide, biochar, and soil properties (Spokas et al., 2009, Cabrera et al., 2011, Trigo et al., 2014, Trigo et al., 2016). However, not all biochar amended soils have resulted in higher sorption (Cabrera et al., 2014, Rittenhouse et al., 2014, Hall et al., 2015).

Sorption coefficients of alachlor increased by a factor of 4 to 33-times in all biochar amended soils as compared to the unamended soil; the greatest sorption was in PA soil amended with wood chip biochar (Table 3). This alteration following the biochar or biomass additions were larger than the differences due to the tillage management.

These trends in sorption are consistent with the chemical and physical properties of the sorbents: sorption of these pesticides tends to increase with increasing organic carbon (Schwab et al., 2006). Sorption of herbicides in soils with differing organic carbon contents is often normalized to the organic carbon content of the soils to obtain K_{oc} values for modeling of herbicide behavior in dissimilar soils. However, it appears that this approach will not work in characterizing sorption in biochar-amended soils versus unamended soils, as K_{oc} values exhibited a trend opposite to that using K_f values (Spokas et al., 2009).

Based on the desorption coefficient (K_d) values, alachlor was considered intermediate desorbing herbicide to the three soils, with K_d ranging 6.61 $\pm$ 0.27 to 62.83 $\pm$ 0.06 L kg^{-1} (Table 3). The sorption was up 94% for wood chips biochar in the amended three soils and desorption was inversely proportional to the sorption, however, only 3% of alachlor was desorption with addition wood chips biochar (Table 3).

The K_{oc}-values did not affect the variability among the three soils, which this variance remained similar with K_d-values, and K_{oc} ranging 308.33 ± 9.80 to 1669.48 ± 36.05 L kg^{-1} in all biochar-amended soils (Table 3). This is justified due to the minimal variation among the properties of the three soils known to commonly affect sorption including OC (Table 1). The highest increase in K_{oc}-values (7 times) in biochar-amended soils in relation to feedstock raw was reported with wood chips in PA, and the smallest increase (~1 time) was reported with soybean residues also in PA (Table 3). The increase in K_{oc}-values at low organic carbon content is due to the increasingly important contribution of mineral phase sorption to the total sorptive capability of the sediment and is evidence for that both sorptive processes are operative, where the K_{oc}-values are 412 ± 37 L kg^{-1} for alachlor (Grundl and Small, 1993).

The OC was the soil attribute that correlated better with alachlor herbicide sorption potentials, but the Fe-oxides also contributed to sorption enhancement in the studied soils. Sorption of alachlor tended to increase under the new harvesting system without straw burning, but it did not change their mobility classification (Giori et al., 2014).

Vermicompost has a higher organic matter content than soil, therefore the CEC values are higher than of soil. This indicates that organic matter from vermicompost contains many interaction sites, and is more likely to react with organic molecules. Isotherm data for alachlor on the matrices studied showed that the vermicompost/soil matrix presents higher adsorption and slightly smaller desorption of alachlor herbicide than soil (Alves et al., 2001). For all three soils evaluated here, the soils possessed an average sorption percentage of 44% and the addition of raw biomass (10% w/w) increased this by 15 to 69% sorbed. On the other hand, biochar increased the sorption to 88%.

Table 3. Distribution coefficients (K_d), sorption coefficient (K_{oc}), and desorption K_d, total sorbed and desorbed (%) for alachlor in amended soils from three states Americans (Minnesota, silt loam; Illinois, silty clay loam; and Pennsylvania, silt loam) with soybean residues, sugarcane bagasse, and wood chips (pine) derived biochars or same raw feedstocks.

Soil	Feedstock	Amended	$K_{d\ (sorption)}$ (L kg^{-1})	$K_{d\ (desorption)}$ (L kg^{-1})	$K_{oc\ (sorption)}$[a] (L kg^{-1})	Sorption (%)	Desorption (%)
MN	Control	-	1.89 ± 0.06[b]	5.30 ± 0.07	76.36 ± 2.64	48.53 ± 0.87	27.40 ± 0.28
	Soybean Residues	Raw	3.72 ± 0.29	7.02 ± 0.36	150.52 ± 12.05	64.45 ± 1.07	22.00 ± 0.89
		Biochar	16.84 ± 0.84	31.43 ± 0.97	681.81 ± 34.08	89.20 ± 0.71	5.98 ± 0.17
	Sugarcane Bagasse	Raw	5.85 ± 0.20	9.03 ± 0.52	237.02 ± 8.28	74.14 ± 0.11	18.57 ± 0.28
		Biochar	13.80 ± 0.78	17.83 ± 0.87	558.79 ± 31.91	87.14 ± 0.36	10.16 ± 0.81
	Wood Chips (Pine)	Raw	5.20 ± 0.34	9.21 ± 0.74	210.52 ± 14.09	71.77 ± 0.74	17.86 ± 0.02
		Biochar	36.70 ± 0.64	62.83 ± 0.06	1485.90 ± 26.23	94.75 ± 0.02	3.08 ± 0.01
IL	Control	-	2.06 ± 0.05	5.73 ± 0.13	74.22 ± 1.82	50.78 ± 0.61	25.88 ± 0.44
	Soybean Residues	Raw	3.66 ± 0.19	7.18 ± 0.44	131.69 ± 6.92	64.11 ± 0.43	21.80 ± 1.05
		Biochar	18.23 ± 0.51	34.78 ± 0.53	655.75 ± 18.53	89.95 ± 0.47	5.57 ± 0.09
	Sugarcane Bagasse	Raw	6.31 ± 0.11	9.46 ± 0.71	226.84 ± 3.97	75.55 ± 0.20	17.46 ± 0.04
		Biochar	8.58 ± 0.27	17.34 ± 0.79	308.53 ± 9.80	80.79 ± 0.07	10.34 ± 0.24
	Wood Chips (Pine)	Raw	5.56 ± 0.29	9.42 ± 0.26	200.09 ± 10.46	73.13 ± 0.44	17.52 ± 0.41
		Biochar	35.86 ± 0.43	58.67 ± 0.22	1289.96 ± 15.72	94.63 ± 0.17	3.30 ± 0.06
PA	Control	-	0.98 ± 0.05	4.57 ± 0.15	50.03 ± 2.91	32.89 ± 1.28	30.43 ± 0.73
	Soybean Residues	Raw	5.01 ± 0.51	6.61 ± 0.27	255.44 ± 26.25	70.95 ± 1.50	23.78 ± 0.01
		Biochar	6.25 ± 0.58	33.76 ± 0.21	318.76 ± 29.86	75.33 ± 1.21	5.61 ± 0.55
	Sugarcane Bagasse	Raw	3.31 ± 0.16	8.11 ± 0.42	168.84 ± 8.31	61.73 ± 0.34	19.79 ± 0.44
		Biochar	16.27 ± 0.18	15.24 ± 0.28	829.99 ± 12.32	88.85 ± 0.96	11.61 ± 0.18
	Wood Chips (Pine)	Raw	4.58 ± 0.51	8.18 ± 0.56	233.51 ± 26.37	69.05 ± 1.75	19.67 ± 1.09
		Biochar	32.72 ± 0.68	51.60 ± 0.89	1669.48 ± 36.05	94.15 ± 0.15	3.82 ± 0.08

[a] $K_{oc} = K_d/(\%\ OC) \times 100$.

[b] Numbers are mean ± standard deviation of the mean (*n* = 2).

3.3. Alachlor mineralization in biochar amended soil

The end products of atrazine and alachlor mineralization are small inorganic molecules such as CO_2, CO, H_20, NH_3, and other compounds (Liu et al., 2002). Trapping $^{14}CO_2$ indicates total degradation of the ring structure of the chemical. Addition of biochar to soil affected the mineralization of alachlor in the PA soil, because all soils with soybean residues, sugarcane bagasse, and wood chips (pine) derived biochars were different of control (without feedstock) and same raw feedstocks (Table 4 and Fig. 2). Also Spokas et al. (2009) found that addition of biochar to soil affected the dissipation of both atrazine and acetochlor.

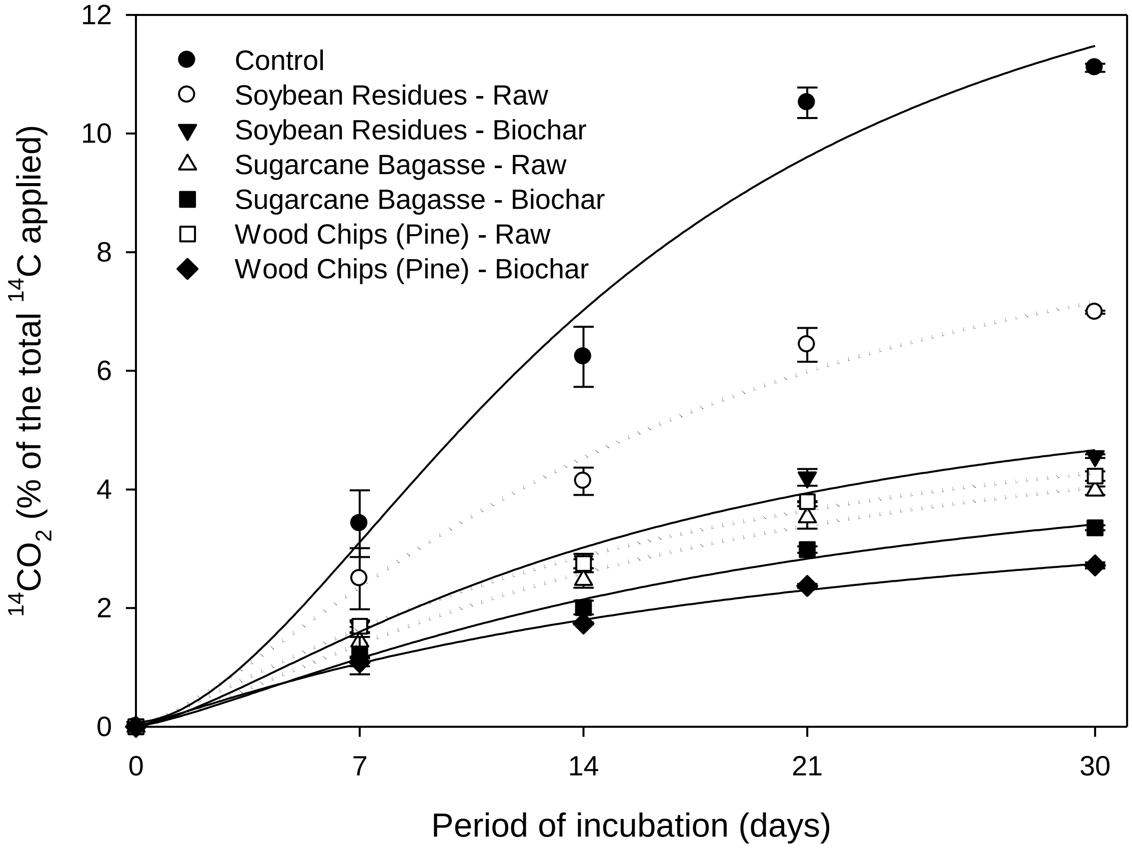

Fig. 2. Alachlor mineralization curve from weekly release of $^{14}CO_2$ (%) during 30 days in silt loam soil (Pennsylvania, USA) with soybean residues, sugarcane bagasse, and wood chips (pine) derived biochars or same raw feedstocks. Vertical bars represent standard deviations (± SD) of means ($n = 2$).

There are statistically significant difference between soils with different feedstock and biochar/raw for alachlor sorption ($p < 0.05$; Tukey's test) (Table 4 and Fig. 2), and there are interaction between each factor alone, where raw soybean residues promoted greater release of $^{14}CO_2$ in relation to other feedstocks, consequently lesser MT_{50} value (~277 d) of alachlor (Table 4). Alachlor mineralization was significantly reduced in soybean residues and wood chip (pine) biochar amended soils as compared to soil amended with the corresponding feedstock. Although similar, there was less $^{14}CO_2$ evolved from sugarcane bagasse biochar amended soil as compared to soil amended with raw feedstock.

Table 4. $^{14}CO_2$ released and accumulated at the 30th day of incubation (%) and parameters of the first order kinetics (mineralization constant rate - k and mineralization half-life - MT_{50}) of the applied ^{14}C-alachlor in silt loam soil (Pennsylvania, USA) with soybean residues, sugarcane bagasse, and wood chips (pine) derived biochars or same raw feedstocks.

Feedstock	Amended	Parameter			
		$^{14}CO_2$ (%)	k (d^{-1})	MT_{50} (days)	R^2
Control	-	11.11 ± 0.56[a]	0.0042 ± 0.00001	165.03 ± 0.54	0.98
Soybean	Raw	6.99 ± 0.51*	0.0025 ± 0.00005	277.25 ± 0.22*	0.98
Residues	Biochar	4.56 ± 0.10*	0.0016 ± 0.00008	433.21 ± 0.18*	0.99
Sugarcane	Raw	3.98 ± 0.25*	0.0014 ± 0.00003	495.10 ± 0.32*	0.99
Bagasse	Biochar	3.35 ± 0.31*	0.0012 ± 0.00002	577.62 ± 0.28*	0.99
Wood Chips	Raw	4.22 ± 0.10*	0.0015 ± 0.00001	462.09 ± 0.13*	0.99
(Pine)	Biochar	2.72 ± 0.07*	0.0009 ± 0.00001	770.16 ± 0.06*	0.99
DMS		2.23		26.80	
CV (%)		12.75		1.76	

[a] The numbers correspond to the mean ($n = 2$) followed by ± standard deviation.

*The asterisk indicates significant differences with respect to the control based on Dunnett's test ($p<0.05$).

In general, the amount of $^{14}CO_2$ accumulated was between 2.7-11.1% in the amended soil with biochars and raw feedstocks (Table 4 and Fig. 2). And then, the addition of wood chips derived biochar was able to increase 4.7X MT_{50} value of alachlor in relation at control soil, without biochar (Table 4). Alachlor mineralization was significantly reduced in all raw feedstock and biochar amended soils as compared to unamended soil.

Munoz et al. (2011) reported that growth of *Candida xestobii* in the presence of ^{14}C-alachlor showed that up to 20% of the ring-labeled compound was mineralized to $^{14}CO_2$ after 48 h. After that time, mineralization proceeded much more slowly, and 40% was transformed after 336 h of incubation. Whereas white rot fungi were previously reported to mineralize the aromatic ring carbon of alachlor to CO_2, with 14% converted to CO_2 after 122 days (Ferrey et al., 1994).

Liu et al. (2002) found an average of 25% of the alachlor applied was mineralized during 56 d laboratory incubation, and total amount of alachlor mineralized and mineralization rate were correlated positively with elevation. Alachlor mineralization rate was higher at the summit than at the other landscape positions, resulting in a shorter half-life at the summit. The authors also reported that alachlor MT_{50} in summit soils averaged about 126 d, whereas MT_{50} was 150 d in backslope/toeslope soils (20% longer resident time).

Although chloroacetanilide herbicides are nonionizable and have moderate to low volatilities (Weber and Peter, 1982), a transparent yellow coloration was observed in the NaOH vial used to trap $^{14}CO_2$ during the first 3 d of the alachlor mineralization experiment. This colored product was not seen in NaOH vials in control uninoculated biometer flasks containing alachlor mineralization studies, corroborating the results of Munoz et al. (2011).

^{14}C-alachlor used in this experiment was ring labeled, therefore any observed effect of biochar on production of $^{14}CO_2$ in amended soil as compared to unamended soil would be the net effect of biochar on the degradation of alachlor and its metabolites. Sorption-desorption would affect the amount of alachlor in solution available to degraders. It is important to remember that herbicide degradation depends on the quantity and activity of degrading soil microorganisms. Degradation also depends of the availability of the herbicide and metabolites to the degraders. Availability of herbicides and metabolites depends on soil sorption-desorption processes; sorption-desorption controls the amount of herbicide and metabolite in solution that would be available to the degrader.

Few studies have examined the influence of other soil-applied organic amendments on herbicide bioavailability (Spokas et al., 2009). Any effect of added organic amendments on herbicide processes in soil including degradation may disappear after a period of incubation of the organic amendment in soil (Delgado-Moreno et al., 2007). More research on the effects of aged biochar residues on herbicide dissipation and weed control is needed.

4. Conclusions

Zonal tillage separates management of row and inter-row positions, while non-zonal tillage applies management uniformly across a field, does not influence alachlor sorption in the soil. The use of biochar as soil organic amendment does ensure greater alachlor sorption-desorption, consequently it decreases alachlor mineralization. The source and amount of organic matter on the biochar can alter alachlor sorption-desorption and mineralization, but these effects can be different for different types of herbicides. Characterization of biochar to be used as a soil amendment is highly recommended prior to field application to optimize sorption conditions and to prevent increased soil and water alachlor contamination following biochar application.

Acknowledgements

The authors would like to thank the São Paulo Research Foundation (FAPESP) (2015/16655-1) for the financial support.

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