Research Article

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Bromoxynil degradation in a Mississippi silt loam soil

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Abstract

BACKGROUND: The objectives of these laboratory experiments were: (1) to assess bromoxynil sorption, mineralization, bound residue formation and extractable residue persistence in a Dundee silt loam collected from 0–2 cm and 2–10 cm depths under continuous conventional tillage and no-tillage; (2) to assess the effects of autoclaving on bromoxynil mineralization and bound residue formation; (3) to determine the partitioning of non-extractable residues; and (4) to ascertain the effects of bromoxynil concentration on extractable and bound residues and metabolite formation.

RESULTS: Bromoxynil K_d values ranged from 0.7 to 1.4 L kg^{-1} and were positively correlated with soil organic carbon. Cumulative mineralization (38.5% ± 1.5), bound residue formation (46.5% ± 0.5) and persistence of extractable residues ($T_{1/2}$ < 1 day) in non-autoclaved soils were independent of tillage and depth. Autoclaving decreased mineralization and bound residue formation 257-fold and 6.0-fold respectively. Bromoxynil persistence in soil was rate independent ($T_{1/2}$ < 1 day), and the majority of non-extractable residues (87%) were associated with the humic acid fraction of soil organic matter.

CONCLUSIONS: Irrespective of tillage or depth, bromoxynil half-life in native soil is less than 1 day owing to rapid incorporation of the herbicide into non-extractable residues. Bound residue formation is governed principally by biochemical metabolite formation and primarily associated with soil humic acids that are moderately bioavailable for mineralization. These data indicate that the risk of off-site transport of bromoxynil residues is low owing to rapid incorporation into non-extractable residues.
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Keywords: biodegradation; mineralization; bound residues; oxidative coupling

1 INTRODUCTION

Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) is a selective post-emergence herbicide used for control of certain broadleaf weeds in corn (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], wheat (*Triticum aestivum* L.), barley (*Hordeum leporinum* L.), oats (*Avena sativa* L.), rye (*Secale cereale* L.), triticale (*Triticale hexaploide* Lort.), conservation reserve program areas, non-residential turfgrass, non-cropland and industrial sites.[1] The herbicide was used for post-emergence broadleaf weed control in transgenic bromoxynil-resistant cotton (*Gossypium hirsutum* L.) and canola (*Brassica napus* L.) until these crops were discontinued.

In spite of many years of bromoxynil use, relatively few papers are available on its fate in soil, whether from laboratory incubations or field studies.[2,3] More information is available on the cometabolism of bromoxynil by various bacteria, which has provided an understanding of its potential transformations in soil. These studies indicated three basic enzymatic mechanisms that initialize the degradation of bromoxynil: (a) nitrile hydratase incorporating water to form the benzamide, as found in *Agrobacterium radiobacter*,[4] *Pseudomonas putida*[5] and *Varivorax* sp.;[6] (b) nitrilase hydrolyzing the nitrile moiety to the benzoic acid derivative, as found in *Klebsiella pneumoniae*;[7] and (c) oxidative displacement of the nitrile group, catalyzed by a flavin monoxygenase (PCP hydroxylase), in *Flavobacterium* sp., forming the hydroquinone derivative.[8] Under anaerobic conditions, bromoxynil and its metabolites are subject to reductive dehalogenation.[9]

The objectives of these laboratory experiments were: (1) to assess ^{14}C-bromoxynil sorption, mineralization, bound residue formation and persistence of extractable residues in native (i.e. non-autoclaved) Dundee silt loam soil collected from 0–2 cm and 2–10 cm depths from plots under 10 years of continuous conventional tillage (CT) and no-tillage (NT) soybean (*Glycine max* L.) production; (2) to compare ^{14}C-bromoxynil mineralization, bound residue formation and persistence of extractable residues between native and non-autoclaved CT 0–2 cm soils; (3) to determine the partitioning of non-extractable residues between the humic and fulvic acid fractions in CT 0–2 cm soil; and (4) to ascertain the effects of bromoxynil concentration (2 or 10 mg kg^{-1})

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on extractable residues, bound residue and metabolite formation in CT 0–2 cm soil.

2 MATERIALS AND METHODS

2.1 Soils

The soils (Dundee silt loam, fine silty, mixed thermic Aeric Ochraqualf) used in this study were collected from long-term soybean research plots with no history of bromoxynil use in Stoneville, Mississippi, maintained under NT or CT for 10 consecutive years.[10] NT plots were not tilled after the fall of 1997. CT plots were tilled each year in the fall after soybean harvest and/or in the spring before planting soybean. CT plots were tilled with a disk harrow and a field cultivator. Soil was collected from four replicate plots of each tillage regime 2 months after soybean harvest at two depth increments (0–2 cm and 2–10 cm) using a 7.5 cm diameter probe. Ten cores from each plot were pooled, passed through a 2.25 cm sieve and stored at 4 °C until used.

2.2 Herbicides

Uniformly ring-labeled ^{14}C-bromoxynil (specific activity = 1092 MBq Mmol^{-1}) was a generous gift of Aventis Corp. (currently Bayer Crop Science). Technical-grade bromoxynil and the benzoic acid metabolite (3,5-dibromo-4-hydroxybenzoic acid) were purchased from Aldrich Corp (Milwaukee, WI).

2.3 Analysis of soil chemical, physical and biological properties

Chemical and physical properties were determined on air-dried soil that was ground through a Wiley mill. Soil textural analysis was done by the hydrometer method.[11] Soil pH was determined on triplicate aqueous suspensions (2 : 1). Total carbon and nitrogen content was determined on duplicate samples using a Flash EA 1112 elemental analyzer (CE Elantec, Lakewood, NJ).

2.4 Bromoxynil mineralization and degradation as affected by tillage and soil depth

Soil (25 g of air-dried weight equivalent) was added to two biometer flasks[12] for destructive termination at 3 and 35 days after treatment (DAT). Duplicate flasks (250 mL polypropylene centrifuge bottles) were also established for each tillage depth replicate and sampled at 0 and 14 DAT to ascertain extractable and non-extractable residues. A solution of ^{14}C-bromoxynil and technical-grade bromoxynil stock was prepared in distilled water to attain a concentration of 25 µg mL^{-1} of bromoxynil and a total radioactivity of 3750 Bq mL^{-1}. A quantity of 2 mL of the stock solution was uniformly applied and additional water was added to attain a soil moisture content of 28%, a bromoxynil concentration of 2 µg g^{-1} and a total radioactivity of 300 Bq g^{-1} soil. Herbicide mineralization was monitored by periodic sampling of sodium hydroxide solution (1 M) in the side-arm traps of the biometer flasks. After sampling, the sodium hydroxide was replaced with fresh solution. ^{14}C-carbon dioxide trapped in the sodium hydroxide was determined on duplicate 1 mL aliquots by liquid scintillation spectroscopy (LSS) (Packard TriCarb 4000 series; Packard Instruments Co., Meriden, CT) using Hi-Ionic scintillation fluid (Packard Instruments), 15 mL per sample. Sampling of biometer flasks was based on the rate of mineralization, daily for the first 3 days, then every 2–4 days during the remainder of the 35 day incubation. Cumulative mineralization data were fitted to the two-compartment model:

$$P = S_{0_1}[1 - \exp(k_1 * t)] + S_{0_2}[1 - \exp(k_2 * t)] \quad (1)$$

where P is evolved ^{14}C-carbon dioxide (%), S_{0_1} and S_{0_2} represent the initial bromoxynil concentration in compartments 1 and 2 respectively (%) and k_1 and k_2 represent the first-order rate constants for compartments 1 and 2 respectively (day^{-1}). Compartments 1 and 2 were defined as the free and bound bromoxynil residues respectively.[13]

The centrifuge bottles were extracted at 0 and 14 DAT, and biometer flasks were extracted at 3 and 35 DAT. The initial extraction used 75 mL of 0.01 M calcium chloride for 2 h to assess readily bioavailable bromoxynil.[14] The second extraction was overnight (20 h) with 75 mL of 80% aqueous methanol, which, based on preliminary studies, was capable of extracting >95% of bromoxynil added to soil. After each extraction, supernatants were collected by centrifugation, and extractable radioactivity was determined on duplicate aliquots using LSS with Ecolume scintillation cocktail (Ecolume; ICN, Costa Mesa, CA). Soils were air dried, and non-extractable radioactivity was determined by oxidation of duplicate 300 mg samples.[15,16]

2.5 Bromoxynil sorption

Batch equilibration was used to assess sorption using air-dried soil from both depths of each plot.[17,18] Solutions were prepared in 0.01 M calcium chloride with ^{14}C-labeled bromoxynil, with appropriate amounts of technical-grade material added to prepare solute concentrations of 12.5, 50 and 100 mg L^{-1}. A quantity of 2 g of soil was added to 25 mL Corex™ centrifuge tubes, 10 mL of solution was added and the tubes were sealed with teflon-lined screw caps. The tubes were shaken for 17 h at 24 °C at 100 rpm in a rotary shaker before termination by centrifugation (10 min at 10 000 × g). The supernatant was removed, and radioactivity was determined on duplicate 1 mL samples mixed in Ecolume scintillation cocktail by LSS. Sorption was described by the average distribution coefficients for the three herbicide concentrations:

$$S = K_d C \quad (2)$$

where S is the amount of herbicide sorbed to soil (µmol kg^{-1}), K_d is the distribution coefficient (L kg^{-1}) and C is the concentration of the two herbicides in solution (µmol L^{-1}).[19]

2.6 Effects of autoclaving

To assess the role of biological/biochemical processes on bromoxynil degradation, the effect of autoclaving was assessed. Soil collected from the surface of CT soil (25 g dry weight equivalents) was placed in biometer flasks, sealed with aluminum foil and autoclaved for 15 min at 137 °C for three consecutive days. Four replicate flasks were established for autoclaved and non-autoclaved soil for a destructive termination at 3 and 14 DAT. Soil was treated with a mixture of ^{14}C-labeled and non-labeled herbicides, as described in Section 2.5, and incubated at 25 °C. Sodium hydroxide was sampled daily for the first 3 days, and then at 5, 7, 10 and 14 DAT. Extraction with calcium chloride followed by methanol and oxidation to determine non-extractable residues were carried out by the protocols described in Section 2.4. The extracts were concentrated by rotary evaporation to remove the methanol, and the remaining aqueous phase was diluted,

acidified and eluted through a Baker C_{18} solid-phase extraction column (Philipsburg, NJ). The column was extracted with 2.0 mL of methanol, evaporated to 1.0 mL.

Non-extractable residues were further fractionated from the non-autoclaved soils only. A quantity of 5 g of air-dried soil was extracted with either potassium phosphate buffer (0.1 M, pH 8.5) or 0.1 M sodium hydroxide for 24 h. The sodium hydroxide extract was acidified with 6.0 M hydrochloric acid to a pH of 1.0 and centrifuged at 12 000 × *g* for 15 min to precipitate the humic acids. Radioactivity recovered in the supernatant after extraction or remaining in solution following acidification was determined via LSS using Hi-Ionic fluor as the scintillation cocktail.

2.7 Effect of bromoxynil concentration

Soil collected from 0–2 cm CT plots (2.0 g dry weight equivalents) was placed in 25 mL Corex glass centrifuge tubes. The soil was treated with a mixture of ^{14}C-labeled and non-labeled bromoxynil solution (0.25 mL) added to attain either 2 or 10 mg kg^{-1}, 1700 Bq g^{-1} and a 25% final moisture content. Soils were extracted twice with 5 mL of methanol at 0, 0.5, 1, 2, 3 and 6 (10 mg kg^{-1}) DAT, radioactivity recovery was determined and the extracts were combined and reduced in volume for radiological high-pressure liquid chromatography (RAD-HPLC) analysis.

2.8 HPLC analysis

Soil extract ^{14}C-analytes were quantified on a Waters 2695 HPLC separation module (Waters Inc., Milford, MA) equipped with a 4.6 by 150 mm C18 SunFire™ column (Waters Inc.) and an in-line 1000 µL liquid flow cell detector (β-ram; IN/US, Tampa, FL). The injection volume was 100 µL, and the flowrate was 0.75 mL min^{-1}. Two mobile phases were used in a gradient program. The initial mobile phase consisting of acetonitrile + water (10 + 90 by volume) was changed during the next 20 min to the final mobile phase consisting of acetonitrile + water (90 + 10 by volume). In-Flow™ 2:1 was used as scintillation fluid at a flowrate of 0.75 mL min^{-1}. Using this methodology, the retention time for bromoxynil was 9.1 min, and for 3,5-dibromo-4-hydroxybenzoic acid 4.3 min.

2.9 Statistical analysis

Soil chemical and physical properties, methanol recovery of radioactivity, mineralization and non-extractable ^{14}C were subjected to analysis of variance (ANOVA) using the general linear model procedure in SAS.[20] Means were separated using Fisher's protected LSD test at $P = 0.05$. Non-linear regression techniques (SAS PROC NLIN) were used to estimate the average K_d with the three herbicide concentrations, and values were fitted by equation (2).

3 RESULTS AND DISCUSSION

3.1 Soil properties

NT 0–2 cm soils contained approximately 72% greater organic carbon and 108% greater total nitrogen compared with the other soils (Table 1). Conversely, similar levels of organic carbon and total nitrogen were observed between NT 2–10 cm and CT 2–10 cm. This level of organic carbon accumulation would be expected in the surface of soils managed under continuous NT conditions for 9 years.[15,21,22] Textural analysis was similar among tillage and depth with mean sand, silt and clay contents of 262, 558 and 180 g kg^{-1} respectively (data not shown). A slightly higher pH was measured in 0–2 cm depths compared with the lower depth of both soils.

Table 1. Soil organic carbon, total nitrogen content and pH of a Dundee soil as affected by tillage and depth[a]

Soil[b]/depth (cm)	Organic carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	pH
NT/0–2	13.31a	1.79a	6.02a
CT/0–2	7.73b	0.86b	6.02a
NT/2–10	9.46b	1.04b	5.82b
CT/2–10	8.70b	0.95b	5.72b
Least significant difference (LSD), $P \leq 0.05$			

[a] Mean of four replicates; means followed by the same letter do not differ at the 95% confidence level.
[b] NT, no tillage, CT, conventional tillage.

Table 2. Bromoxynil sorption in two depths of soil from no-tillage (NT) and conventional tillage (CT) Dundee silt loam soils. Linearized distribution coefficient (K_d) and organic carbon distribution coefficient (K_{oc}) values calculated on three concentrations[a]

Tillage	Depth (cm)	Bromoxynil K_d (L kg^{-1})	Bromoxynil K_{oc} (mL g^{-1})
NT	0–2	1.35 (±0.26)	75 (±6)
CT	0–2	0.72 (±0.03)	91 (±10)
NT	2–10	0.87 (±0.14)	92 (±12)
CT	2–10	0.78 (±0.13)	91 (±3)
Least significant difference (LSD), $P \leq 0.05$		0.25	14

[a] Means and standard deviation of four replicates for each concentration and soil collected from four replicate blocks.

3.2 Bromoxynil fate as influenced by tillage and depth in soil profile

Bromoxynil sorption was higher in NT 0–2 cm compared with the other soils and was positively correlated with soil organic carbon (Table 2). The K_d values observed for bromoxynil in the Dundee silt loam are similar to those reported in the literature.[6,23] The lower K_{oc} in NT 0–2 cm compared with the other soils is consistent with the greater abundance of aged organic matter that differed in quality for sorption of bromoxynil. The sorption studies indicate a relatively moderate sorption potential for bromoxynil compared with other herbicides,[17–19] and that the relative organic matter content and perhaps the nature of the organic matter contribute to the bromoxynil sorption.

Regardless of tillage or depth, there were no differences in mineralization kinetics among treatments (Fig. 1). Bromoxynil mineralization kinetics was similar to that reported for a German silt loam.[24] The initial fast mineralization kinetics followed by slower kinetics for all soils suggests rapid incorporation of the herbicide residues into soil components with a low bioavailability, as predicted by the two-compartment model.

The initial recovery of ^{14}C-bromoxynil 4 h after treatment with 0.01 M calcium chloride ranged from 24% in NT 0–2 cm soil to 52% in CT 0–2 cm soil, while greater bromoxynil was recovered with methanol compared with the other soils (Table 3). On the other three sample dates, less than 1 and 3% of the radioactivity applied was recovered in calcium chloride and methanol extracts respectively, regardless of tillage or soil depth. A rapid decrease in calcium chloride extraction indicates that a small

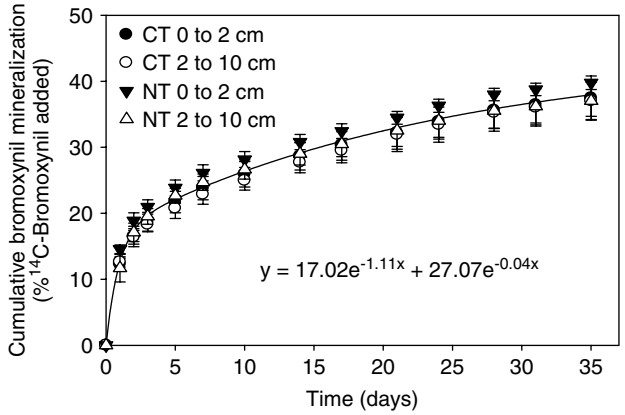

Figure 1. Cumulative mineralization of ^{14}C-bromoxynil in two depths of a Dundee silt loam soil from conventional tillage (CT) and no-tillage (NT) plots.

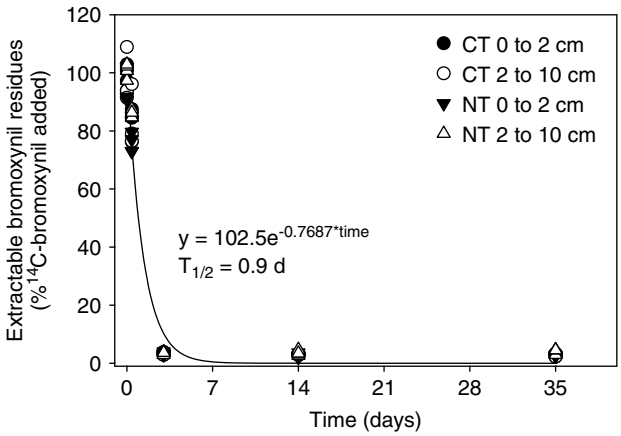

Figure 2. Total extractable ^{14}C-bromoxynil residues ($CaCl_2$ and methanol combined) in two depths of a Dundee silt loam soil from conventional tillage (CT) and no-tillage (NT) plots.

amount of the bromoxynil residues is available for immediate microbial metabolism, similarly to glyphosate.[14] The low amount of radioactivity recovered by extraction prohibited discrimination of parent compound and metabolites using radiological HPLC analysis. This current research with a Dundee silt loam indicates a rapid degradation with a half-life of <1 day (Fig. 2), regardless of tillage or depth. In recent studies, Baxter and Cummings[25] observed a bromoxynil half-life of approximately 5 days. These authors did not report on the characteristics of the soil or the incubation conditions for the study. At 3 days following application, 69–75% of the bromoxynil residues were associated with non-extractable soil components (Table 3). The radioactivity associated with the non-extractable fraction decreased with each sample, with ~44–47% remaining in the non-extractable fraction following incubation for 35 days. The decline in non-extractable residues corresponded to the lower amount of mineralization observed between 3 and 35 DAT, as suggested by the two-order mineralization model.

3.3 Effect of autoclaving on bromoxynil degradation

Similar mineralization rates were observed in native soil as in the previous study, but mineralization was completely inhibited in autoclaved soil (Table 4). Throughout the incubation, more than 50% of the ^{14}C-bromoxynil applied was recovered in the initial aqueous calcium chloride extraction, while 1.0% or less of the ^{14}C was recovered with calcium chloride from the non-autoclaved soils. Methanol extraction recovered an additional 30% of the applied ^{14}C in autoclaved soil, while 6% or less was recovered with methanol from non-autoclaved soil at 3 and 14 DAT. Subsequently, more than 58% of the ^{14}C-bromoxynil was associated with non-extractable residues in non-autoclaved soil, compared with 4% in autoclaved soil. HPLC analysis of soil extracts from the autoclaved

Table 3. Recovery of radioactivity in two depths of soil from no-tillage (NT) and conventional tillage (CT) soils treated with ^{14}C-bromoxynil[a]

		Recovery (% of initial ^{14}C-bromoxynil applied)				
Time (days)	Soil/depth (cm)	$CaCl_2$-extractable	Methanol-extractable	Cumulativemineralized	Non-extractable	Total recovery
0.17	NT/0–2	23.6c	52.0a	0	19.3a	95.0a
	CT/0–2	52.0a	33.7b	0	15.1b	100.8a
	NT/2–10	41.2b	40.8b	0	15.0b	97.1a
	CT/2–10	42.8b	39.2b	0	15.1b	97.0a
3	NT/0–2	0.8a	2.5a	21.5a	68.8a	94.0a
	CT/0–2	0.9a	2.5a	18.7ab	71.1a	93.2a
	NT/2–10	0.8a	2.7a	17.9b	74.7a	96.1a
	CT/2–10	0.9a	2.6a	18.3b	71.2a	93.0a
14	NT/0–2	0.3a	2.5a	31.1a	50.4a	84.0a
	CT/0–2	0.3a	2.6a	28.7b	53.6a	85.2a
	NT/2–10	0.5a	3.0a	29.0ab	51.5a	84.0a
	CT/2–10	0.3a	2.7a	27.7b	54.2a	84.9a
35	NT/0–2	<0.1b	2.6a	39.8a	43.9a	86.3a
	CT/0–2	<0.1b	2.4a	37.5a	46.6a	86.5a
	NT/2–10	0.4a	2.6a	37.0a	46.5a	86.5a
	CT/2–10	<0.1b	3.0a	37.0a	44.2a	84.3a

[a] Mean of four replicates; means followed by the same letter do not differ at the 95% confidence level.

Table 4. Recovery of ^{14}C-labeled bromoxynil from autoclaved and non-autoclaved Dundee silt loam soil

Day	Treatment	Radioactivity recovered[a] (% of ^{14}C-bromoxynil applied) Mineralized	$CaCl_2$-extractable	MeOH-extractable	Non-extractable
3	Autoclaved	<0.1	56.0	36.6	7.0
	Non-autoclaved	15.1	1.0	6.0	63.0
	LSD 0.05	2.0	3.7	3.5	4.0
14	Autoclaved	<0.1	51.7	34.6	9.6
	Non-autoclaved	25.7	0.6	3.7	57.9
	LSD 0.05	1.5	3.9	2.8	3.9

[a] Mean of four replicates.

Table 5. Fractionation of ^{14}C-bound bromoxynil residues from non-autoclaved soil at 3 and 14 DAT[a]

Treatment	Bound residues recovered (% of ^{14}C originally present) 3 days	14 days
Potassium phosphate (pH 8.5)	18.7(±1.5)	9.3(±2.1)
Sodium hydroxide (0.1 N)	57.9(±2.8)	60.7(±3.2)
Acid precipitated from NaOH extraction	40.2(±2.5)	52.4(±2.1)

[a] Mean and standard deviation of four replicates.

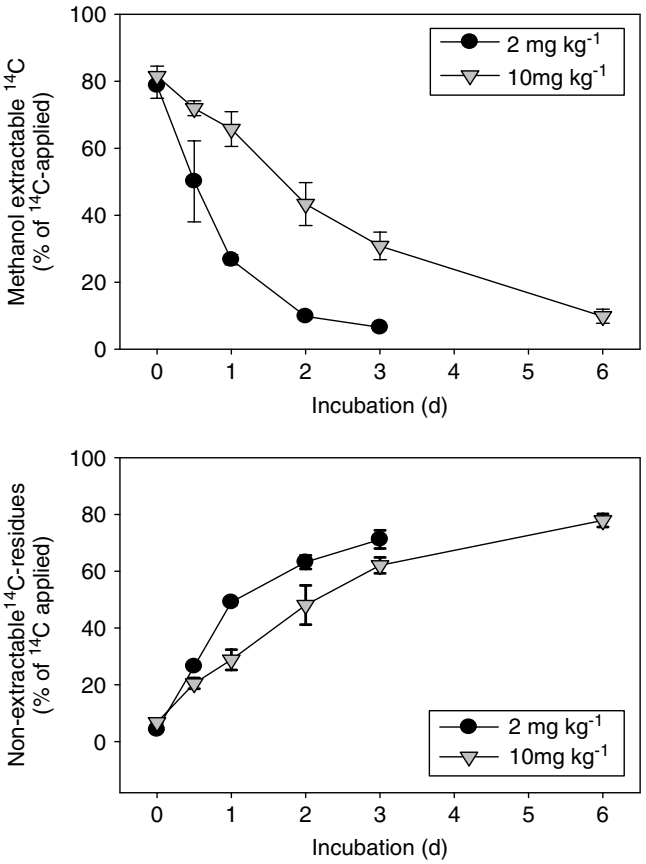

Figure 3. Methanol-extractable ^{14}C-bromoxynil residues and non-extractable residues from a Dundee silt loam treated with 2 mg kg^{-1} bromoxynil (circles) and 10 mg kg^{-1} bromoxynil (triangles). Mean and standard deviation of four replicates.

soil at 3 and 14 days indicated that 100% of the radioactivity was recovered as bromoxynil. However, concentrated extracts from native soil were so degraded that resolution of parent herbicide and metabolites was not feasible using RAD-HPLC analysis. These data suggest that either bromoxynil or its degradate(s) are rapidly sequestered into soil organic matter via a biological or biochemical process. Secondly, these results indicate that, although there is potential for sorption to soil colloids, the physical sorption equilibration process has little impact on the formation of bound non-extractable bromoxynil residues.

Multiple extraction techniques were used to characterize the nature of the non-extractable ^{14}C-residues from non-autoclaved soil from the autoclaving study (Table 5). When 0.1 M sodium hydroxide was used as extractant, ~60% of the non-extractable residues was extracted. When the sodium hydroxide extracts were acidified to pH 1.0, the humic acids precipitated and the solution was clarified by centrifugation.[26] In soils incubated for 3 DAT, 69% of the ^{14}C dissolved with sodium hydroxide was precipitated with the humic acids. However, in soils incubated for 14 DAT, 86% of the ^{14}C dissolved with sodium hydroxide was precipitated with the humic acids. The ^{14}C remaining in solution after acidification of the sodium hydroxide extracts should be associated with the fulvic acid fraction.[26] When slightly alkaline phosphate buffer (pH 8.5) was used as extractant, much less of the ^{14}C residues was extracted, 19 and 9% in soils that were incubated for 3 and 14 days respectively. A similar amount of radioactivity was associated with the fulvic acid fraction and the potassium-phosphate-extractable ^{14}C-residues. A mixed-mode extraction technique using slightly alkaline buffer has been used by others to recover hydroxyl atrazine from soils;[27] however, the mild alkaline extractant utilized in this experiment was unable to recover bound bromoxynil residues.

Various chlorinated phenols can be bound into humic polymers via a process called oxidative coupling, which is catalyzed by enzymes such as peroxidases, laccases and monophenol oxidases.[28] No autopolymerization was observed when bromoxynil was incubated alone in the presence of *Rhus vernificera* laccase at pH 4.5 (data not shown), as all the bromoxynil added to the reaction was recovered as the parent compound. However, certain halogenated phenols require the presence of other humic acid precursors, e.g. syringic acid, for oxidative polymerization to occur.[28]

3.4 Effect of bromoxynil concentration on dissipation and metabolite accumulation

When applied at 2 mg bromoxynil kg^{-1} soil, only 27% of the applied radioactivity was extractable within 1 day, and less than 7% was recovered at 3 DAT (Fig. 3a). Extractable bromoxynil residues were more persistent at the higher concentration, with 66 and 31% of the initially applied radioactivity recovered by methanol extraction at 1 and 3 days after application respectively. However, 1.46 mg kg^{-1} of bromoxynil residues was dissipated within 24 h when applied at 2 mg kg^{-1}, compared with 3.4 mg kg^{-1} in 24 h when applied at 10 mg kg^{-1}. Thus, dissipation of bromoxynil residues increased twofold, with a fivefold increase in concentration, indicating that the higher concentration is closer to saturation. However, bromoxynil degradation is a

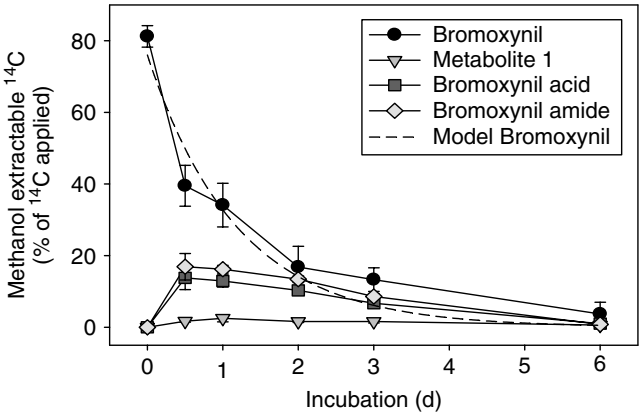

Figure 4. Recovery of bromoxynil and three metabolites from a Dundee silt loam receiving 10 mg kg^{-1} bromoxynil. Mean and standard deviation of four replicates. Dotted line represents predicted dissipation.

multistep process governed by patterns of metabolite formation, sorption and formation of bound residues by oxidative coupling. As reported in the previous two experiments, the decline in extractable ^{14}C residues corresponded to a concurrent increase in non-extractable ^{14}C-residues (Fig. 3b).

The half-life for bromoxynil dissipation at either the 2 or 10 mg kg^{-1} soil rate was $<$1 day (Figs 3a and 4), which was comparable with that reported for two Danish surface soils.[23] However, dissipation rates in the Dundee silt loam soil were several times more rapid than the 6.4 day half-life reported by Baxter and Cummings[25] in an English silt soil with high organic matter content that was treated with 10 mg kg^{-1} bromoxynil. These studies also indicated that, when applied at 50 mg kg^{-1}, there was a long lag before bromoxynil degraded, with a half-life of 28 days. In a Canadian clay soil, the half-life of bromoxynil when applied at 25 mg kg^{-1} was 14 days at 25 °C.[2] Both of these studies used air-dried soils, and this may have been one of the contributing factors to a longer half-life.

The pattern of metabolite accumulation and dissipation of the parent herbicide in soil extracts is presented in Fig. 4. Within 12 h after treatment, RAD-HPLC analysis indicated the appearance of two dominant metabolites with retention times of 4.1 min (metabolite 2) and 5.3 min (metabolite 3) as opposed to the parent at 9.3 min. Metabolites 2 and 3 had a maximum accumulation of 13 and 17% of the radioactivity applied respectively, and were less than 2% of the applied ^{14}C at 6 DAT. In addition, two minor peaks representing $<$2.5% of the total radioactivity added were observed at 2.3 and 17.3 min respectively, with the metabolite of the longer RT formed during the later sample times (3–6 days, data not shown). Using this LC with photodiode array detection methodology, the acid derivative of bromoxynil had a retention time of 3.9 min, which corresponded to metabolite 2 with a RAD-LC peak of 4.1 min. Based on prior studies, metabolite 3 is most likely the amide derivative;[2,24] unfortunately, the authors do not have access to this metabolite. The rapid formation and dissipation of multiple metabolites corresponding to the formation of bound residues signifies that the microbial formation of metabolites is most likely required for bound residue formation. In recent research by Nielsen *et al.*,[6] it was suggested that persistent transformation products of bromoxynil may be formed. These current studies indicate that, although several metabolites are formed, these metabolites are actually less persistent than the parent, and may present only a limited risk in Mississippi soils.

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