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Earthworm burrowing and feeding activity and the potential for atrazine transport by preferential flow

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Abstract

Soil columns with established earthworm burrow structures were subjected to 14 and 47 mm h^{-1} rainstorms to study the effects of *Lumbricus terrestris* L. burrowing and feeding activity on preferential atrazine transport in soil. Earthworm treatments for the soil columns were as follows: earthworms introduced 1 d prior to herbicide application, earthworms introduced 9 d after herbicide application and no earthworms added following herbicide application. Rainfall was applied at 9, 18, 29, 40 and 51 d following [U-ring-^{14}C]atrazine applications onto crop residues at the soil surface. The concentration of radioactivity in leachate was greatest during the first rainfall simulation and decreased in the subsequent four simulated rainfall simulations. Preferential herbicide transport through earthworm burrows was observed during all rainfall simulations, but total atrazine and metabolites in leachate at the end of the five rainfall simulations were approximately 2-fold greater in the absence than in the presence of earthworms. Earthworm feeding activity reduced the potential for herbicide leaching by ingesting and transporting herbicide residues away from the soil surface and increasing the amount of non-extractable (non-leachable) herbicide residues in the soil.

Keywords: Preferential flow; Earthworm feeding activity; Rainfall; Atrazine leaching

I. Introduction

The herbicide atrazine [2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine] is used in North America to control annual broadleaf and grassy weeds in corn and other field crops. Although most of the applied atrazine remains in the upper soil layer, its off-site contamination of surface and groundwater has been well documented (Goodrich *et al.*, 1991; Liu *et al.*, 1996). Atrazine has a greater mobility in soils than many other herbicides and may persist in aquatic environments for many years (Buhler *et al.*, 1993).

Atrazine is retained by both inorganic and organic colloid surfaces, but organic matter is more adsorbant than clay (Mersie and Foy, 1986). Atrazine transformation involves hydroxylation, dealkylation and ring cleavage, requiring chemical and microbial processes. The transformation products, deisopropylatrazine and deethylatrazine, have a greater mobility in soils than atrazine because their compound adsorption potential is smaller (Adams and Thurman, 1991; Sorenson *et al.*, 1994). Hydroxyatrazine (pK_a of 5.1) is adsorbed more strongly on soil than atrazine (pK_a of 1.7), because its protonation is greater at the same soil pH (Clay and Koskinen, 1990).

Atrazine has been detected in groundwater following the first major rainstorm after its application to agricultural fields (Gish *et al.*, 1991; Isensee and Sadeghi, 1995). This indicates that water infiltrating into the soil may follow preferred pathways and bypasses other parts of the soil matrix. Preferential flow paths result in more rapid movement of water and solutes than would be estimated by models based in the classical

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infiltration theory (i.e. Darcian flow).

The earthworm *Lumbricus terrestris* L. is an important contributor to develop preferential flow paths in soils because it tends to create vertical burrows which are open at the surface and may extend to a depth greater than 3 m (Lee, 1985). With more water infiltrating into soils through vertical *L. terrestris* burrows, there is a potential for non-point source entry of atrazine into groundwater (Sigua *et al.*, 1995).

The rate of water and herbicide transport through *L. terrestris* burrows is influenced by various factors. Less atrazine is transported through earthworm burrows when there is a delay before the first significant rainfall after herbicide application (Edwards *et al.*, 1993). Low intensity storms following herbicide application may move surface-applied atrazine into the soil matrix thereby reducing herbicide losses via burrow flow in subsequent high intensity storms (Shipitalo *et al.*, 1990). However, atrazine transport via burrow flow has been demonstrated to occur under low intensity irrigation, i.e. < 7 mm h^{-1} (Sigua *et al.*, 1995), perhaps via saturated flow through smaller macropores. Water repellency at the soil surface increases herbicide transport via burrow flow by limiting infiltration into the soil matrix at the onset of rainfall (Shipitalo and Edwards, 1996). Undisturbed burrows in no-till fields conduct more water and herbicides than those truncated by tillage (Isensee *et al.*, 1990; Sadeghi and Isensee, 1992). Herbicide adsorption onto organic-rich burrow linings can decrease the amount of atrazine that is preferentially transported through the soil profile in no-till fields (Stehouwer *et al.*, 1993, 1994).

Earthworms can move organic material from the soil surface to depth (Darwin, 1881; Edwards and Heath, 1962; Shipitalo *et al.*, 1988). A *L. terrestris* population of 40 to 60 m^{-2} in an alfalfa no-till field incorporated 26% of surface residues into the soil during 30 d in the Spring (Gallagher and Wollenhaupt, 1997). Farenhorst *et al.* (2000) found that *L. terrestris* feeding on atrazine-sprayed crop residues also move herbicides from the soil surface to depth and reduce the concentration of herbicides at the soil surface. Earthworm feeding activity during interstorm periods may therefore influence the potential of atrazine transport via burrow flow in subsequent rainstorms. Our aim was to examine the effects of *L. terrestris* feeding activity on preferential atrazine transport in soil.

2. Material and methods

2.1. Soil, earthworms and crop residues

The Gobles soil (gleyed brunisolic gray brown luvisol) in Ontario was sampled (0 to 15 cm) in a no-till corn field. Prior to the experiment, the soil contained no detectable atrazine residues (HPLC detection limit 25 ng g^{-1} soil). Key physical and chemical soil properties of the soil include: 29% sand, 46% silt, 24% clay, 1.63% organic carbon, pH 6.35, CEC 12.38 cmol kg^{-1} and an exchangeable K, Ca, Mg and Na of 0.47, 5.62, 1.56 and 0.07 cmol kg^{-1}, respectively. Soil texture was determined by the hydrometer method (Gee and Bauder, 1986), organic C by the modified Walkley-Black volumetric oxidation method (Allison, 1965), pH in a 1:1 soil to 10 mM $CaCl_2$ solution, CEC by the NH_4OAC method (Chapman, 1965) and exchangeable K, Ca, Mg and Na according to Jackson (1958).

Corn leaves were taken from mature plants on a pesticide-free field plot at the Southern Crop Protection and Food Research Centre, London, Ontario. Moistened leaves were held in plastic bags at room temperature for 1 month to decompose and stimulate food acceptance by earthworms (Edwards and Lofty, 1977). Subsequently, leaves were air-dried and pulverized (2.5-7.5 mm^2) using a blender to produce a material more palatable for earthworms. Corn leaves were used in clearing earthworm digestive tracts (see below) and in column experiments (see Section 2.3).

Mature earthworms *(L. terrestris)* were purchased locally and stored in environmental growth chambers at 12°C. Worm digestive tracts were cleared of their ingested contents by keeping them in soil containing corn leaf residues for 5 d before they were used in experiments.

2.2. Chemicals

A commercially-available atrazine-metolachlor liquid formulation, Primextra® (label content: 153 g atrazine L^{-1}, 364 g metolachlor L^{-1} and 10 g unidentified other active triazines L^{-1}; Ciba-Geigy Co., Greensboro, NC) was diluted with reverse osmosis-purified water. [U-ring-^{14}C]Atrazine (97% radiochemical purity; sp. act. 14.8 x 10^7 Bq $mmol^{-1}$; Novartis Crop Protection Canada, Mississauga, Ont.) was used in experiments to determine the mass balance and chemical identity of atrazine transformation products. Herbicide stock solutions for experiments were prepared by mixing the [U-ring-^{14}C]atrazine with the diluted Primextra® to give a final radioactive concentration of 13 x 10^3 Bq ml^{-1} and total atrazine concentration of 35 µg ml^{-1}.

The chemical identity of herbicide transformation products was determined by high-performance liquid chromatography (HPLC) as described in Section 2.5 and using the following analytical standards: atrazine, hydroxyatrazine, deethylatrazine, deisopropylatrazine, deethyldeisopropylatrazine, deethylhydroxyatrazine, deisopropylhydroxyatrazine and deethyldeisopropylhydroxyatrazine (all > 97% purity; Ciba-Geigy Co., Greensboro, NC).

2.3. Soil column preparation, earthworm introduction and experimental design

For the main experiment, a series of PVC columns (24 cm long and either 6.3 or 5.1 cm i.d.) were prepared with one earthworm per column to establish burrow structures in soil. Earthworms were fed on moistened corn residues (approximately 0.5 g week^{-1}, based on air-dried leaf weight) for 6 months. Following earthworm removal as described in Farenhorst *et al.* (2000), soil columns were saturated from below and drained for 5 d to reach field capacity. Earthworm burrows were very stable and did not collapse during the saturation procedure. The number of burrow openings at both the soil surface and the base of columns was used as a measure to divide columns into groups, with five replicates in each group (see below). Earthworms removed from columns were not used in further experiments and released in nature.

Fifteen of the prepared soil columns with 5.1 cm i.d. were grouped (by the number of burrow openings per column) into 3 earthworm treatments x 5 replicates; these columns are referred to as those receiving 14 mm h^{-1} simulated rainfall. Ten of the prepared soil columns with 6.3 cm i.d. were grouped (by the number of burrow openings per column) into 2 earthworm treatments x 5 replicates; these columns are referred to as those receiving 47 mm h^{-1} simulated rainfall.

For the 10 columns receiving 47 mm h^{-1} rainfall, one treatment received one earthworm (4-5 g) per column, 1 d prior to atrazine application and no earthworms were added to the second treatment. Averaged over five columns (for each of the two treatments), there were 4.6 burrows on the surface (2.9% of the total soil surface) and 2.8 burrows (1.8% of the total soil surface) on the bottom of columns. The standard deviation of burrows was somewhat greater on the surface (2.1 and 2.7, with and without worms, respectively) than at the bottom of the cores (0.8 and 1.5, with and without worms, respectively).

For the 15 columns receiving 14 mm h^{-1} rainfall, one treatment received one earthworm (4-5 g) per column, 1 d prior to atrazine application, the second treatment received one earthworm (4-5 g) per column at 9 d after atrazine application and atrazine was applied but no earthworms were added to the third treatment. Averaged over five columns (for each of the three treatments), there were 5.2 burrows on the surface (5.0% of the total soil surface) and 2.8 burrows (2.7% of the total soil surface) on the bottom of columns. The standard deviation of burrows was similar for all three treatments at the bottom of the cores, 0.8, and ranged from 1.1 to 1.9 at the soil surface.

2.4. Herbicide and rainfall applications

Surface corn leaves (1 g) were added onto the soil surface just prior to herbicide applications. Herbicide solutions were uniformly applied onto the surface corn leaves by pipette. Each column received 94 µg unlabeled atrazine and 113 µg of [U-ring-^{14}C]atrazine (77 x 10^{3} Bq). Subsequently, each column was stored in a separate container with NaOH traps for CO_2 and kept for 68 d in environmental growth chambers at 12°C. NaOH traps were removed and replaced at 1, 2, 10, 22, 46 and 68 d following atrazine application.

Water (5 mM $CaSO_4$ solution) was applied using a constructed drip rainfall simulator, designed to deliver steady and evenly distributed rainfall at various intensities. For each column, rainfall (0.78 mm) was applied on soil columns at 9, 18, 29, 40 and 51 d following herbicide applications. Thus, the total amount of rainfall received over five rainfall simulations was 3.90 mm per column. For each rainfall simulation, rainfall duration was 10 min for the 47 mm h^{-1} and 33 min and 20 s for the 14 mm h^{-1} rainfall. The rainfall intensities and durations were selected to represent frequently recurring natural rainstorms in Southern Ontario. For example, a rainfall-intensity-duration frequency diagram for the area around Belmont (field soil site), shows a 2-yr return period for 47 and 14 mm h^{-1} rainstorms with a duration of 30 and 120 min, respectively (AES, 1994).

During rainfall applications, a stainless steel mesh (1 mm) was placed over the soil surface to reduce raindrop impact and distribute rain more evenly. Columns were set on a funnel to facilitate the leachate collection in glass vials (15 ml). Glass vials were replaced every 2 min for the 47 mm h^{-1} and every 6 min and 40 s for the 14 mm h^{-1} rainfall, corresponding to a water application of 0.16 mm. After simulated rainfall was terminated, columns were covered with a glass plate and ‘subsequent leachate’ was collected for 20 min in a glass vial.

The leachate was quantified by weight and the radioactivity in the leachate was quantified by liquid scintillation counting (LSC). Samples were analysed by HPLC with an ultraviolet absorption detector (UV) and radioactivity detector (RD) to quantify and identify residual parent compounds and metabolites.

At the conclusion of the experiment (68 d after atrazine application), representative soil samples were taken from 0 to 8, 8 to 12 and 12 to 20 cm depths in three of five replicated columns for each treatment. Total, non-extractable and extractable radioactivity were determined as described below.

2.5. Analytical methods

Herbicide mineralization was determined by trapping $14CO_2$ in a scintillation vial containing 5 ml of 1 N NaOH. Representative soil samples (duplicates) were methanol-extracted twice with a soil-methanol ratio of 1:2 (v/v) and a shaking time of 30 min at 30°C, then supernatants from both extractions were pooled. Radioactivity in methanol extracts and NaOH traps was determined by LSC using 10 ml of UniverSol Scintillation Cocktail (ICN, Costa Mesa, CA) and correcting for quenching with an external standard.

The total radioactivity was determined by combusting soil (0.5 g) for 4 mM in a Model OX 300 biological oxidizer (R.J. Harvey Instrument Corp., Hillsdale, NJ). The $^{14}CO_2$ was trapped in Carbon-14 Scintillation Cocktail (R.J. Harvey Instrument Corp., Hillsdale, NJ) and counted by LSC. A recovery efficiency of 97% was determined by combusting a standard quantity of radioactivity. The amount of non-extractable (bound) atrazine at each depth was estimated by subtracting the amount of extractable radioactivity from the total radioactivity. Lyophilized earthworms (incl. gut contents) were also combusted and the recovered radioactivity quantified to obtain a complete mass balance on ring-^{14}C. A 5 to 10 g subsample of soil matrix was used to determine soil moisture content (w/w).

The radioactivity in the leachate was determined by adding 100 µl subsamples (duplicates) in plastic vials with 10 ml scintillation cocktail and counting by LSC. For some samples, the remaining water was evaporated in tubes under air-flow. Herbicide residues in tubes were then dissolved in 1 ml methanol and samples were filtered (0.5 gm). Radioactive atrazine and metabolites in methanolic solutions were quantified by reverse phase HPLC with UV (Waters 490 Programmable Multi-wavelength Detector, Waters Chromatography Division, Milford, MA) and radioactivity (Berthold Model LB506 C-1, Berthold Instruments, Pittsburgh, PA) detection. Instrument operating conditions were as follows: column: reversed-phase, 25.0 cm x 4.6 mm (10 p.m Partisil 10 ODS-3 packing); mobile phase: methanol-10 mM ammonium acetate (50:50) at 1 ml min^{-1}; detector wavelength: 220 nm. The fraction of ^{14}C in parent compound and in metabolites was quantified by integrating peak areas obtained with the radioactivity detector.

2.6. Data analysis

Data was analysed using SigmaStat® Windows Version 1.0 (Jandel Scientific). For the 15 columns receiving 14 mm h^{-1} rainfall, treatments were compared by analysis of variance and multiple comparison (Student-Newman-Keuls-test). For the 10 columns receiving 47 mm h^{-1} rainfall this was done by an unpaired t-test. All statements of significance are at the $P < 0.05$ level.

3. Results

Earthworms introduced 1 d prior or following [U-ring-^{14}C]atrazine applications inhabited previously established burrow structures for survival in columns, and did not develop additional burrows in soil.

For each drainage sample, the total amount of radioactivity in drainage was quantified by counting the total radioactivity in the sample and subtracting the 80 dis min^{-1} background value. Atrazine residue concentrations in leachate were expressed as dis min^{-1} ml^{-1}, as some samples did not contain sufficient radioactivity to quantify the fraction of ^{14}C in the parent compound and metabolites by HPLC-RD analysis. For these samples, only the total amount of radioactivity in drainage could be measured (atrazine + metabolites) and it was not possible to convert this to mass units (µg of atrazine or µg of atrazine metabolites).

3.1. Atrazine distribution and dissipation

At the conclusion of the experiment, more than 80% of the initially applied radioactivity remained in the soil, after both high and low intensity rainfall simulations. For some soil layers, the amount of non-extractable atrazine residues was significantly greater in soils with earthworms compared with no-earthworm soils (Tables 1 and 2). Generally, the amount of non-extractable radioactivity in soils with earthworms was similar in the 0 to 8 cm and 8 to 12 cm layers but less in the 12 to 20 cm soil layer. The amount of non-extractable atrazine residues also decreased with depth in no-earthworm soils.

For the surface soil layer (0-8 cm), a significantly greater amount of radioactivity remained methanol-extractable in no-earthworm soils compared with soils containing earthworms (Tables 1 and 2). Generally, the amount of extractable radioactivity in no-earthworm soils was greater in the 0 to 8 cm layer than in the 8 to 12 cm and 12 to 20 cm soil layer, after both 47 and 14 mm h^{-1} rainfall simulations. In soils with earthworms, extractable radioactivity in the 0 to 8 cm layer was between 11 and 14% and less than extractable radioactivity found at 8 to 12 and 12 to 20 cm depths.

Less than 2% of the initially applied [U-ring-^{14}C] atrazine was mineralized, independent of the earthworm treatment and simulated rainfall intensities. On average, 6% of the initially applied radioactivity was detected in *L. terrestris.* No significant differences were observed between initial and final (at 68 d after atrazine applications) earthworm weights indicating that earthworms were not adversely affected by herbicide applications. Total recovery (leachate + column, inclusive earthworm + mineralization) of the initially applied radioactivity in soils ranged between 95 and 102%.

Table 1. Distribution of radioactivity in soil after five rainfall simulations at 47 mm h^{-1} for soils containing earthworm burrows with or without active earthworms.

Earthworm treatment	Soil layer		
	0-8 cm	8-12 cm	12-20 cm
Extractable radioactivity			
Earthworms introduced before herbicide application	13.6[a] ± 1.7 x[b]	23.2[a] ± 1.8 x[b]	19.4[a] ± 2.6 x[b]
No earthworms	39.4 ± 2.6 y	18.3 ± 1.5 x	21.6 ± 2.5 x
Non-extractable radioactivity			
Earthworms introduced before herbicide application	12.3[c] ± 1.5 x[b]	14.5[c] ± 1.4 x[b]	7.9[c] ± 1.3 x[b]
No earthworms	7.8 ± 1.3 x	6.9 ± 1.2 y	4.7 ± 1.1 x

[a] Mean extractable radioactivity in soil layer as % of initially applied radioactivity.

[b] Means (± standard error) followed by the same letters are not significantly different (*n* = 6; for each earthworm treatment 3 of the 5 replicated columns were sampled and 2 samples (duplicates) were taken for each soil layer and each column).

[c] Mean non-extractable radioactivity in soil layer as of % of initially applied radioactivity.

3.2. Atrazine transport via burrow flow

On average, approximately 60% of applied water leached through soils (with a 5% open burrow density at the surface) receiving 14 mm h^{-1} rainfall and 33% of the applied water percolated through soils (with a 2.9% open burrow density at the soil surface) receiving 47 mm h^{-1} rainfall (Figs. 1A and 2A); the remaining portions drained from columns between rainfalls.

For all columns, radioactivity was detected in the first leachate during the first of the 47 and 14 mm h^{-1} rainfall simulations. The total radioactivity in some of these samples was as much as 0.2% of the initially applied [U-ring-^{14}C]atrazine. Farenhorst (1998, unpublished Ph.D. thesis, University of Toronto) subjected columns without earthworm burrows to similar rainfall simulations and found no radioactivity in leachate until the third rainstorm. Therefore, we concluded that the rapid breakthrough of radioactivity in our study was a result of preferential flow.

For both 47 and 14 mm h^{-1} rainfall, the average concentration of radioactivity in leachate decreased with each succeeding rainfall (Figs. 1B and 2B). Combining the results from five rainfall simulations, cumulative radioactivity leached was slightly greater for soils (with a 5% open burrow density at the surface) receiving 14 mm h^{-1} rainfall than for soils (with a 2.9% open burrow density at the soil surface) receiving 47 mm h^{-1} rainfall (Figs. 2C and 1C).

3.3. Effects of earthworm feeding activity on preferential atrazine transport

Earthworms rapidly moved corn residues from the soil surface into the soil. For the treatments in which *L. terrestris* was added to soil columns 1 d prior to atrazine applications, approximately 90% of the initially applied corn residues were incorporated into the soil prior to the first rainfall simulation at 9 d following herbicide applications. For the treatment in which earthworms were added to columns after the

Table 2. Distribution of radioactivity in soil after five rainfall simulations at 14 mm h^{-1} for soils containing earthworm burrows with or without active earthworms.

Earthworm treatment	Soil layer		
	0-8 cm	8-12 cm	12-20 cm
Extractable radioactivity			
Earthworms introduced 1 d before herbicide application	11.3[a] ± 1.2 x[b]	23.5[a] ± 1.4 x[b]	17.1[a] ± 2.2 x[b]
Earthworms introduced 9 d after herbicide application	12.4 ± 1.2 x	22.6 ±2.5 x	20.2 ± 2.4 x
No earthworms	38.2 ±2.4 y	19.4 ± 2.3 x	19.6 ± 1.5 x
Non-Extractable radioactivity			
Earthworms introduced 1 d before herbicide application	16.2[c] ± 1.4 x[b]	12.7 [c] ± 1.3 x[b]	5.3[c] ± 1.1 x[b]
Earthworms introduced 9 d after herbicide application	11.2 ± 1.4 xy	10.3 ±1.2 xy	9.2 ± 1.3 x
No earthworms	6.1 ± 2.2 y	4.4 ± 1.1 y	3.9 ± 1.1 y

[a] Mean extractable radioactivity in soil layer as % of initially applied radioactivity.

[b] Means (± standard error) followed by the same letters are not significantly different (*n* = 6; for each earthworm treatment 3 of the 5 replicated columns were sampled and 2 samples (duplicates) were taken for each soil layer and each column).

[c] Mean non-extractable radioactivity in soil layer as of % of initially applied radioactivity.

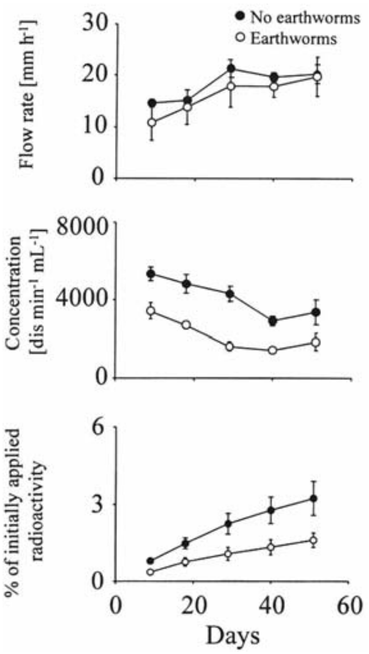

Fig. 1. Leachate from soil columns with earthworm burrows either without or containing active earthworms during 47 mm h^{-1} rainfall simulations. Each point in graphs corresponds to a simulated rainfall event and is an average of 5 replicated columns. (A) Average drainage rate for each rainstorm, (B) average concentration of radioactivity in drainage for each rainstorm and (C) total radioactivity in drainage for each and previous rainstorms.

first rainfall simulation, earthworms moved approximately 90% of the initially applied corn residues into the soil prior to the second rainfall simulation at 18 d following herbicide applications.

The presence of earthworms in soil significantly decreased the concentration of radioactivity in leachate when compared with no-earthworm soils, at both 14 and 47 mm h^{-1} rainfall intensities. For all rainfall simulations at 47 mm h^{-1}, no-earthworm soils showed 1.8 x more radioactivity ml^{-1} leachate than soils with earthworms (Fig. 1B). For the first rainfall simulation at 14 mm h^{-1}, leachate through no-earthworm soils showed 1.2 x more radioactivity ml^{-1} than soils with earthworms (Fig. 2B). Adding earthworms to soil after the first rainfall simulation influenced the concentration of radioactivity in leachate in the second and subsequent rainfall simulations. Leachate from no-earthworm soils showed 1.8 x more radioactivity ml^{-1} during the second and subsequent rainfall simulations compared with leachate from soils with earthworms introduced at 9 d after herbicide applications.

The quantity and identity of transformation products in leachate were indistinguishable for soils with or without earthworm activity and at both 14 and 47 mm h^{-1} simulated rainfall (Tables 3 and 4). In the first simulated rainfall, atrazine was the dominant compound (~80%) with minor leaching of hydroxyatrazine (~20%). Deethylhydroxyatrazine was the predominant compound in leachate during subsequent rainfall simulations, with minor leaching of hydroxyatrazine and deethylatrazine. The portion of leached radioactivity found as metabolites in leachate of soils increased from about 50 to about 70% in the second and the third rainfall simulations, respectively, while more than 90% was found in leachate of soils in the fourth and fifth rainfall simulation.

Combining the results of all five 47 mm h^{-1} rainfall simulations, the total accumulated radioactivity in leachate was significantly greater for no-earthworm soils (3.7% of initially applied radioactivity) compared with soils containing earthworms (1.7% of initially applied radioactivity) (Fig. 1C). Combining the results of all five 14 mm h^{-1} rainfall simulations, the total cumulative radioactivity in leachate was significantly greater for no-earthworm soils (4.1% of initially applied radioactivity) compared to the two treatments containing earthworms (Fig. 2C). The cumulative radioactivity in leachate was statistically similar for soils with earthworms introduced prior to herbicide

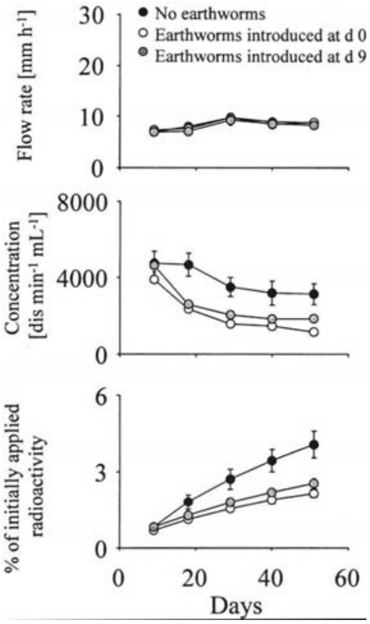

Fig. 2. Leachate from soil columns with earthworm burrows either without or containing active earthworms during 14 mm h^{-1} rainfall simulations. Each point in graphs corresponds to a simulated rainfall event, and is an average of 5 replicated columns. (A) Average drainage rate for each rainstorm, (B) average concentration of radioactivity in drainage for each rainstorm and (C) total radioactivity in drainage for each and previous rainstorms.

Table 3. Percentage of atrazine and its metabolites in drainage of soils containing earthworm burrows during five 47 mm rainfall simulations at 9, 18, 29, 40 and 51 d after [U-ring-^{14}C]atrazine application. Soils were treated with or without active earthworms.

Days	Atrazine (%)	Metabolites (%)
Earthworms introduced before herbicide application		
9	83 ± 2[a]	17 (OA)[b]
18	53 ± 5	47 (OA and DEA)
29	36 ± 8	64 (DEOA > OA and DEA)
40	ND[c]	ND
51	ND	ND
No earthworms introduced following herbicide application		
9	89 ± 4	11 (OA)
18	51 ± 6	49 (OA and DEA)
29	30 ± 10	70 (DEOA > OA)
4	< 10	> 90 (DEOA > OA)
51	< 10	> 90 (DEOA > OA)

[a] Mean (± standard error) percentage of atrazine in drainage as of percentage of total radioactivity in drainage.

[b] Mean percentage of metabolites in drainage as of percentage of total radioactivity in drainage. Identity of metabolites: OA = hydroxyatrazine, DEA = deethylatrazine, DEOA = deethylhydroxyatrazine.

[c] ND = radioactivity in samples was too low to determine the identity of the herbicide residues.

application and soils with earthworms introduced at 9 d following herbicide applications, this was 2.2 and 2.5% of the initially-applied radioactivity, respectively.

4. Discussion

The amount of radioactivity in leachate from soils was greatest in the first rainstorm, then decreased with each succeeding rainfall event. This indicated that [U-ring-^{14}C]atrazine residues moved deeper into the soil matrix, reducing the availability of radioactivity at the soil surface and its potential to enter burrows during subsequent rainfall simulations. The retention of [U-ring-^{14}C]atrazine residues by the soil matrix would also have increased through time, reducing the potential for transport through the soil profile.

The predominant [U-ring-^{14}C]atrazine residues in leachate were atrazine and the metabolites deethyl-

Table 4. Percentage of atrazine and its metabolites in drainage of soils containing earthworm burrows during 14 mm rainfall simulations at 9, 18, 29, 40 and 51 d after herbicide application. Soil were treated with or without active earthworms.

Days	Atrazine (%)	Metabolites (%)
Earthworms introduced before herbicide application		
9	86 ± 3[a]	14 OA > DEOA[b]
18	49 ± 7	51 DEOA > (OA and DEA)
29	32 ± 8	68 DEOA > OA
40	ND[c]	ND
51	ND	ND
Earthworms introduced 9 d after herbicide application		
9	82 ± 4	18 OA
18	45 ± 4	55 DEOA > (OA and DEA)
29	39 ± 4	61 DEOA > OA
40	< 10	> 90 DEOA > OA
51	ND	ND
No earthworms introduced following herbicide application		
9	85 ± 4	15 OA
18	52 ± 6	48 DEOA > OA
29	31 ± 5	69 DEOA > OA
40	< 10	> 90 DEOA > OA
51	< 10	> 90 DEOA > OA

[a] Mean (± standard error) percentage of atrazine in drainage as of percentage of total radioactivity in drainage.

[b] Mean percentage of metabolites in drainage as of percentage of total radioactivity in drainage. Identity of metabolites: OA= hydroxyatrazine, DEA = deethylatrazine, DEOA = deethylhydroxyatrazine.

[c] ND = radioactivity in samples was too low to determine the identity of the herbicide residues.

hydroxyatrazine and hydroxyatrazine. Leachate samples contained less deethylatrazine and no other atrazine metabolites. Given the nature of the metabolites in the leachate, dechlorination followed by dealkylation was probably the most important pathway of atrazine degradation in the Gobles soil, or these metabolites were more mobile than others that may have been produced. In other column studies, deethylatrazine and deisopropylatrazine were found at greater depth than other atrazine degradation products, including deethylhydroxyatrazine and hydroxyatrazine (Kruger *et al.*, 1993). Sorenson *et al.* (1994) argued that the presence of hydroxyatrazine at depth was a result of *in situ* atrazine degradation rather than leaching. Our study indicated that hydroxyatrazine may move to depth by preferential transport through earthworm burrows.

Preferential herbicide transport through soils was less with, than without earthworms. For the soils with a 2.9% open burrow density, this was due to the combined effects of a greater concentration of radioactivity in leachate and a greater flow rate in no-earthworm soils, compared with soils containing earthworms. Soils with earthworms exhibited a slightly slower average leachate rate than no-earthworm soils because corn residues, transported into burrows by earthworms and the presence of earthworms themselves obstructed burrow flow. For the soils with a 5% open burrow density, the greater earthworm burrow volume in soils diminished the differences in leachate rates between soils with and without earthworms. In these soils, the cumulative radioactivity in leachate was only affected by the concentration of radioactivity in leachate which decreased in the order of no-earthworm soils > soils with earthworms introduced at 9 d after herbicide application > soils with earthworms introduced 1 d prior to herbicide application.

Earthworms decreased the concentration of herbicides in leachate due to feeding on atrazine-sprayed crop residues, reducing the concentration of atrazine in the surface soil and, therefore, its availability for preferential transport. Because earthworms increased amounts of soil-bound herbicide residues in the soil, as was found by Meharg (1996) and Farenhorst *et al.* (2000), the potential for atrazine transport through the soil profile was accordingly reduced since strongly sorbed herbicides are less likely to leach.

It has been demonstrated that 'wash-off' of herbicides from crop residues could lead to major herbicide losses by surface runoff or by preferential flow paths (Felsot *et al.*, 1990). We suggest that the amount of herbicide leaching through burrows will be related to the extent of previous earthworm feeding activity between rainstorms. Reduced earthworm feeding activity between herbicide applications and the occurrence of major rainstorms will increase the potential for preferential herbicide transport to groundwater via earthworm burrows. Earthworm feeding activity may be reduced when: (1) herbicide applications have adverse effects on earthworms, (2) earthworms avoid herbicide-sprayed crop residues or (3) adverse soil moisture and temperatures in the soil surface layer force earthworms to move deeper. Alternatively, with increasing time between herbicide applications and major rainstorms, earthworm feeding activity removes herbicides from the soil surface to depth and increases the persistence of herbicides in the root zone by enhancing herbicide sorption throughout the soil. In this case, preferential herbicide transport in earthworm burrows will be small.

Our results indicated that preferential herbicide transport via burrow flow to groundwater may be less significant than is usually assumed. Most studies have overestimated herbicide transport via earthworm burrows because (1) experiments were conducted using artificial pores without lining (Czapar *et al.*, 1992), (2) authors based their conclusions on experiments using non-sorbed tracers (Zachman *et al.*, 1987; Zachman and Linden, 1989) and (3) rainfall was applied soon after herbicide application, ignoring the influence of earthworms on herbicide dissipation during interstorm periods (Sigua *et al.*, 1993, 1995). For a more realistic assessment of herbicide dissipation and movement in soils with earthworm burrows, laboratory studies using intact soil columns should include the native earthworm population. Studies using packed columns should contain introduced earthworms long enough to allow for adequate development of burrow linings.

In this study, less than 2.5% of the initially applied [U-ring-^{14}C]atrazine leached through the 20 cm deep soils containing burrows and earthworms. Steenhuis *et al.* (1990) reported that 0.1% leaching of a 2 kg ha.$^{-1}$ chemical application can result in concentrations greater than 1 µgL^{-1} in groundwater. However, atrazine movement in field soils would be normally less than was observed in our study. For example, the number of earthworm burrows openings at the soil surface of columns corresponded to a 2.9 and 5% surface area, which is greater than that generally reported for no-till soils (Ehlers, 1975; Edwards *et al.*, 1988). Shipitalo *et al.* (1990) reported that the density of burrow openings at the base of large no-till soil blocks corresponded to only a 0.58% surface area. In our study, approximately half of the burrows in columns were continuous with depth, corresponding to 1.5 and 2.5% of the area of the bases of the columns. Also, the processes which generally reduce the downward movement of herbicides in field soils were not included, i.e. wetting and drying cycles and evaporation. Wetting and drying cycles in surface soil can increase atrazine sorption and decrease its transport potential (White, 1976) and the evaporation of water from the surface of field soils can retard the downward movement of herbicides (Ma and Selim, 1996).

5. Conclusion

The amount of preferential herbicide transport during rainfall in soil columns containing earthworm burrows was dependent on the presence or absence of active earthworms during interstorm periods. After five rainfall simulations of either 14 or 47 mm h^{-1} on these soils, total atrazine and metabolites in leachate was approximately 2-fold greater with, than without earthworms. Earthworm feeding activity reduced the potential for herbicide leaching by ingesting and transporting herbicide residues away from the soil surface, and increasing the amount of non-extractable (non-leachable) herbicide residues in the soil. From the results our study, we suggest that atrazine, applied to no-till soils at recommended rates, would be minimally leached under normal Ontario weather conditions, unless there is a heavy rainfall shortly after application. After earthworms have moved surface-applied herbicides into the surficial layers, burrows may be transmitting relatively clean rain water past the herbicide-containing soil matrix. In this case, water transported through burrows would be less contaminated with atrazine than water moving down through the soil matrix.

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