



Dissipation of triclosan, triclocarban, carbamazepine and naproxen in agricultural soil following surface or sub-surface application of dewatered municipal biosolids



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HIGHLIGHTS

- We characterized the soil fate of four organic contaminants carried in biosolids.
- Biosolids were placed on the soil surface or incorporated within the soil profile.
- Naproxen, triclosan and triclocarban were dissipated more rapidly when incorporated.
- Depth of placement did not influence the rate of carbamazepine dissipation.
- Soil incorporation of biosolids will result in more rapid dissipation of contaminants.

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ABSTRACT

In many jurisdictions land application of municipal biosolids is a valued source of nutrients for crop production. The practice must be managed to ensure that crops and adjacent water are not subject to contamination by pharmaceuticals or other organic contaminants. The broad spectrum antimicrobial agents triclosan (TCS) and triclocarban (TCC), the anti-epileptic drug carbamazepine (CBZ), and the nonsteroidal anti-inflammatory drug naproxen (NAP) are widely used and are carried in biosolids. In the present study, the effect of biosolids and depth of placement in the soil profile on the rates of TCS, TCC, CBZ, and NAP dissipation were evaluated under semi-field conditions. Aggregates of dewatered municipal biosolids (DMBs) supplemented with ¹⁴C-labeled residues were applied either on the soil surface or in the subsurface of the soil profile, and incubated over several months under ambient outdoor conditions. The dissipation of TCS, TCC and NAP was significantly faster in sub-surface than surface applied biosolid aggregates. In contrast the dissipation rate for CBZ was the same in surface applied and incorporated aggregates. Overall, the present study has determined a significant effect of depth of placement on the dissipation rate of biodegradable molecules.

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1. Introduction

Municipal biosolids contain a wide range of pharmaceuticals and personal care products (PPCP) that persist and that partition into the organic fraction during the wastewater treatment process (McClellan and Halden, 2010; USEPA, 2009). Many jurisdictions permit the use of biosolids as a source of crop nutrients and valued soil conditioner in commercial agriculture (O'Connor et al., 2005; Requirements for

application of prescribed materials, 2013; Service Ontario eLaws, 2014). Following application to land, there is potential for transport of PPCP residues to adjacent surface or shallow groundwater through surface runoff, leaching, or preferential flow (Edwards et al., 2009; Gottschall et al., 2012; Topp et al., 2008b). There is also a potential for crop uptake of PPCPs, although management practices such as a one-year delay between application and harvest of crops for human consumption result in negligible uptake (Prosser et al., 2014; Sabourin et al., 2012). Contamination of water or crops with PPCP residues is of concern with respect to human and environmental health (Boxall et al., 2012).

The persistence of PPCP residues in soil following biosolid application is the key factor limiting the opportunity for the contamination of

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Table 1

Structure and key properties of the chemicals evaluated in this study (Chu and Metcalfe, 2007; Sabourin et al., 2009; Ying et al., 2007).

Compound CAS # formula	M.W	Solubility (mg L ⁻¹)	Log K _{ow}	pKa	Structure
Triclosan 3380-34-5 5-chloro-2-(2,4-dichlorophenoxy)-phenol	289.5	4.621	4.7	8.1	
Triclocarban 101-20-2 3,4,4'-trichlorocarbanilide	315.6	0.6479	4.9	n/a	
Carbamazepine 000298-46-4 5H-dibenzo[b,f]azepine-5-carboxamide	236.3	17.7	2.45	14	
Naproxen 022204-53-1 (2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid	230.3	15.9	3.18	4.15	

crops or adjacent water. Persistence of a PPCP is governed by its inherent recalcitrance, soil composition and physical and chemical properties, and climate factors such as moisture and temperature. Another key set of factors are the composition of the biosolids PPCP residues are entrained in, and how they are applied and incorporated into soil. Biosolids vary widely in their moisture content, ranging from slurry (liquid municipal biosolids; LMBs) with over 95% moisture content to dewatered cake (dewatered municipal biosolids; DMBs) with typically about 70% moisture content. The former is handled and applied as a liquid, whereas the latter is applied as a solid. The LMB is typically surface applied followed by incorporation, or injected directly into the soil profile (Topp et al., 2008b). The DMB is typically surface applied followed by incorporation, but equipment exists (Terratec Environmental Ltd., dewatered biosolids direct injection system) that can deposit extruded DMB directly into the soil profile (Edwards et al., 2009).

In previous semi-field studies we found the application matrix to have very significant effects on the dissipation kinetics and pathways of triclosan (TCS) and triclocarban (TCC) added directly to soil in water, carried in LMB, or carried in DMB (Al-Rajab et al., 2009). These two biocides are typically carried in biosolids at mg per kg dry weight concentrations (McClellan and Halden, 2010; Sabourin et al., 2012; USEPA, 2009). The dissipation of ¹⁴C-triclosan (5-chloro-2-(2,4-dichlorophenoxy)-phenol; Table 1), was found to be significantly more persistent when carried in aggregates of dewatered biosolids applied to the soil surface, than when incorporated into soil via application of either liquid municipal biosolids or water (Al-Rajab et al., 2009). In contrast, ¹⁴C-triclocarban (3,4,4'-trichlorocarbanilide; Table 1) was found to be equally persistent regardless of how it was applied to soil (Al-Rajab et al., 2009). It was hypothesized that TCS is amenable to biodegradation by soil microorganisms and therefore incorporation promoting contact with soil was a key factor in the kinetics of dissipation. In contrast, TCC is inherently more recalcitrant to biodegradation, and therefore dissipation will not be influenced by the degree of incorporation and enhanced soil contact.

The present study sought to further explore the effect and significance of soil–biosolids contact on experimentally determined persistence kinetics and pathways. In a typical DMB application, the bulk of the aggregates is incorporated within the soil profile by tillage within 24 h of application. Inevitably some DMB aggregates will be left on the soil surface where they are vulnerable to weathering and precipitation-driven surface runoff. We thus evaluated the impact of surface versus sub-surface aggregate placement on PPCP persistence using radioisotope methods. In addition

to ¹⁴C-TCS and ¹⁴C-TCC, we evaluated the dissipation of ¹⁴C-carbamazepine (5H-dibenzo(b,f)azepine-5-carboxamide; CBZ; Table 1) and ¹⁴C-naproxen (2-(6-methoxy-2-naphthyl)propionic acid; NAP; Table 1). The anti-seizure medication CBZ is a commonly prescribed drug for the treatment of epilepsy, and is used in the treatment of bipolar disorder (WHO, 2013). In Canada, an estimated 21–25 tons are prescribed annually (McLaughlin and Belknap, 2008). The nonsteroidal anti-inflammatory drug NAP is widely used for the treatment of pain and swelling associated with arthritis, gout, and other inflammatory conditions. In Canada, an estimated 53–62 tons are sold annually (McLaughlin and Belknap, 2008). Based on previous experience, we anticipated that CBZ would be persistent, and NAP rapidly dissipated in soil under permissive conditions (Li et al., 2013; Topp et al., 2008a).

Our specific objectives in the present study were to: 1. Determine the impact of surface or subsurface soil placement of DMB aggregates on the dissipation kinetics of several PPCPs that commonly occur in biosolids (TCS, TCC, CBZ, and NAP) in outdoor conditions. 2. By using radioisotope methods, measure various dissipation pathways including non-extractable residue formation. 3. Determine if the rate of dissipation in surface-applied aggregates was rate-limited by mass loss through weathering.

2. Materials and methods

2.1. Chemicals

Triclosan, triclocarban, carbamazepine, naproxen, 3,4-dichloroaniline and 3-chloroaniline, carbamazepine 10,11-epoxide, radiolabeled TCS (4'-chloro-2'-hydroxyphenyl-UL-¹⁴C; purity >99%; specific activity 74 MBq mmol⁻¹), radiolabeled TCC (3,4-dichlorophenyl-ring-UL-¹⁴C; purity >99%; specific activity 828.8 MBq mmol⁻¹), and radiolabeled CBZ (Carbamazepine-carbonyl-¹⁴C; purity >99%; specific activity 836.2 MBq mmol⁻¹) were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Radiolabeled NAP (naproxen[O-methyl-¹⁴C]; purity >99%; specific activity 2035 MBq mmol⁻¹) was purchased from ARC (St. Louis, MO, USA). Methyltriclosan (Me-TCS; 5-chloro-2-(2,4-dichlorophenoxy)anisole) was purchased from Wellington Laboratories Inc. (Guelph, ON). Stock solutions of ¹⁴C-labeled (final radioactive concentrations were 10.14; 12; 19.01 and 9.15 KBq 100 μL⁻¹ for TCS, TCC, CBZ and NAP respectively) and unlabeled (1 mg mL⁻¹) chemicals were prepared in methanol and stored at 4 °C until used in experiments.

2.2. Soil and biosolids

Experiments were undertaken using a sandy loam Brunisolic Grey Brown Luvisol typical of southwestern Ontario (Embro series) soil (0–20 cm depth) obtained from the Agriculture and Agri-Food Canada (AAFC) research farm located in London, Ontario, Canada (42°59'N, 81°15'W). The soil was obtained from an area under sod, had never come in contact with biosolids or manures, and had the following properties: sand:silt:clay (75:19:6); pH 7.3; % organic matter 3.6; cation exchange capacity 13.2 meq 100 g⁻¹. Soils were sieved to a 2 mm maximum particle size, and stored at 4 °C prior to experimentation. Dewatered municipal biosolids (DMBs, centrifuged digested sludge from the plant belt press) were obtained from the Greenway pollution control plant (London, ON). The sewage treatment plant services approximately 350,000 citizens. The DMB had the following key properties: pH 7.78; % organic matter 17; carbon:nitrogen ratio (C:N) 6:1; % dry matter 21.5. The DMB used in the experiments reported here was analyzed for various organic residues using USEPA method 1694 (Axys Analytical Services Inc., Sydney BC, Canada) and contained (in µg g⁻¹) 6.53 of TCS, 3.3 of TCC, 0.125 of CBZ and 0.198 of NAP.

2.3. Field soil core experiments

A series of amended soil cores was incubated on the grounds of the AAFC London Research Farm. The experiment was designed to evaluate the effect of depth of placement of dewatered municipal biosolids on TCS, TCC, CBZ, and NAP fate under ambient environmental conditions. Because radiolabeled parent compounds were employed, procedures were implemented to avoid contaminating adjacent soil or water with radioactivity. The experimental protocol was designed to normalize the soil core temperature with that of the surrounding ground, expose the soil to ambient temperature and precipitation, and minimize the risk of core flooding and contamination of the surrounding area.

The cores were prepared using 5 cm diameter plastic sleeves (PETG; polyethylene terephthalate glycol; Concord Environmental Equipment, Hawley, MN) cut to 15 cm lengths. The bottom end of each sleeve was capped with a fragment of vinyl window screen fixed in place with duct tape. The sleeves were therefore not free draining. The sleeves were filled with soil, and gently tapped to settle the soil to a uniform depth of 13 cm for the surface treatment, and half-filled for the subsurface treatment.

Dewatered municipal biosolids were amended with ¹⁴C-labeled and unlabeled compound stocks to give a final concentration in DMB of 0.83 KBq g⁻¹ of ¹⁴C-labeled compound and 10 µg g⁻¹ of unlabeled compound (in addition to the investigated compounds residues that were in the biosolids at the time of sampling). Thus taking into account the concentrations in biosolids prior to amendment (see above), the aggregates initially contained (in µg g⁻¹) 16.53 of TCS, 13.3 of TCC, 10.13 of CBZ and 10.20 of NAP. Methanol was present in the final DMB preparation at concentrations of 17.3; 16.1; 13.6 and 18.1 µL g⁻¹ following amendment with TCS, TCC, CBZ, and NAP, respectively. After amendment, DMB was homogenized using a stomacher (Smasher, AES Laboratories, Montréal, QC, Canada) at the normal setting for 10 min. Homogenized DMB was packed into a 60 cm³ plastipak syringe (Becton Dickenson, Fisher Scientific, Ottawa, ON) which had the end removed with a razor blade. The DMB was slowly extruded from the syringe barrel and cut into 10 mL portions (corresponding to approximately 10 g “wet weight”) using a pallet knife. The nominal dimensions of each DMB aggregate were 2.5 in length by 2 cm diameter. A single DMB aggregate was placed either on the top of filled cores (surface treatment) or placed on the surface of half-filled cores, that were then carefully topped up to 13 cm depth with soil in order to eliminate any potential dead spaces (subsurface treatment). Columns were then placed in a plastic pail containing water at a depth of 5 cm and allowed to fully saturate from the bottom up. This ensured that volumetric soil water content was uniform across all cores at the start of the experiment and air

bubbles were more efficiently removed from each core. Following saturation, the bottoms of the cores were tightly sealed with duct tape. Cores were then transported to the field and placed in 5 gallon buckets containing a 5 cm layer of pea gravel, and 10 cm layer of sandy loam soil. The buckets were placed in the ground such that the top of the cores were approximately 1 cm above the surrounding soil surface. The buckets were then filled with sandy loam soil such that the cores were surrounded by soil to approximately 1 cm below the top of the core. At each sampling time, triplicate cores from each treatment were removed, taken into the laboratory, placed in ziplock bags that were sealed, and frozen at -7 °C until they could be extracted. The gaps left in the supporting soil by the removed cores were backfilled with soil.

In parallel incubations, the rate of decay of DMB aggregates placed on the soil surface due to weathering was determined. The DMB was prepared exactly as described above except it received 15 µL g⁻¹ methanol only rather than any of the PPCP stock solutions. The cores for this experiment were prepared in the same way as described above, and in addition a disk of vinyl window screen (mesh size 1 mm) cut to the same internal diameter as the soil core liner was placed on the soil surface each core. A single aggregate of DMB was placed on the top of the screen. The cores were saturated from the bottom and transported to the field as described above. At each sampling time, five cores were removed and taken into the laboratory. Each aggregate of DMB was transferred carefully to an aluminum weigh boat, weighed, and dried for 24 h at 105 °C in an oven. The aggregate was then reweighed to estimate the dry mass of DMB.

Meteorological data was obtained on site at the AAFC research farm, using a Campbell Scientific MetData1 weather station (Campbell Scientific, Edmonton, AB, Canada). Soil temperature was obtained using soil thermistor probes attached to the MetData1 station. Total precipitation during the experiment was 299.3 mm. Key environmental and climate conditions during the experiment are presented in Fig. 1.

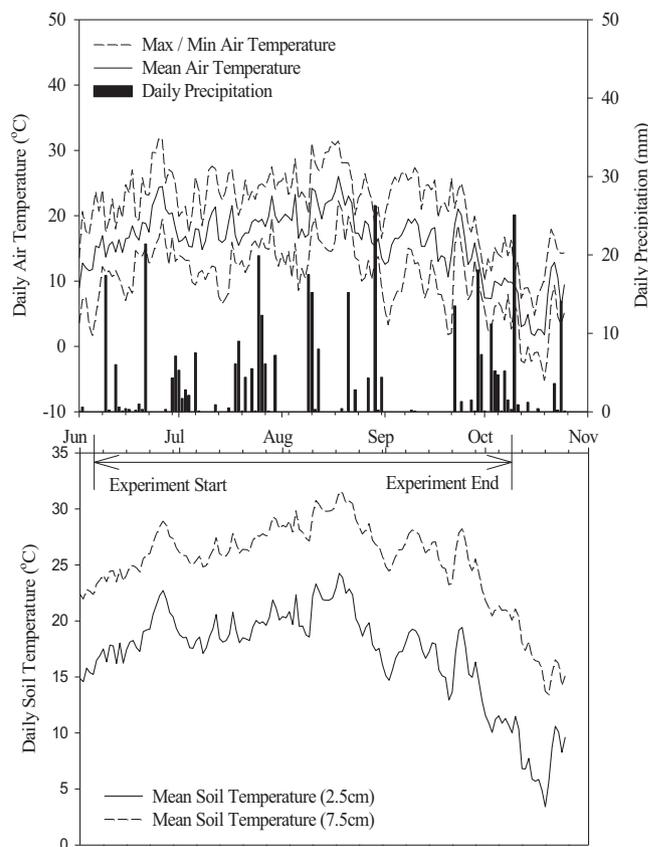


Fig. 1. Weather conditions at the AAFC London, Ontario research farm during the period of the field experiment.

2.4. Extraction of soils

In preliminary experiments, the efficacy of methanol for extraction of the investigated compounds from DMB was established as follows. A 2-g portion of DMB was supplemented with a stock solution of the compound prepared in methanol to give a final radioactive concentration of 0.83 KBq g^{-1} of ^{14}C -labeled compound and a concentration of $10 \mu\text{g g}^{-1}$ of unlabeled compound. The amended DMB was added to a scintillation vial containing 2 g of sandy loam soil (first adjusted to a moisture content of 15%) and supplemented with 16 ml of methanol. These were securely closed, agitated for 10 min on a wrist action shaker, and centrifuged for 10 min at 3000 rpm. Soil was sequentially extracted a further 2 times, the supernatants pooled, and a portion counted by liquid scintillation counting as described below. The efficiency of extraction for the method described above was 88.7 ± 8.7 , 84.3 ± 9.3 , 89.9 ± 9.2 and $83.8 \pm 3.9\%$ for TCS, TCC, CBZ and NAP, respectively.

In order to account for all radioactivity that might have leached through the cores during the experiment, frozen soil cores were partially thawed and cut into three sections of equivalent length, each of which was extracted. This was done by extruding the core out of the bottom of the soil core liner and cutting three uniform lengths using a pallet knife. Sections were collected in individually labeled 1 lb polyethylene bags. Extracts of soil sectioned from the cores that were amended with the investigated compounds applied in DMB were prepared as follows. The entire section was placed into a 250 mL Teflon® centrifuge bottle and a volume of methanol (4 times the sample volume) was added. Bottles were closed securely and agitated for 10 min on a wrist action shaker. Samples were then centrifuged for 10 min at 3000 rpm in a Sorvall SLA-1500 rotor in a Sorvall RC-6-Plus centrifuge (Fisher Scientific, Ottawa). The supernatant was then decanted and filtered by a VWR filter paper to a clean 1000 mL glass Pyrex round bottom bottle (Fisher Scientific, Ottawa). Soil samples were subjected to two more rounds of extraction as described above and all extracts were pooled into the same bottle for that particular sample. The extract was dried down to 10 mL using a rotary evaporator (Buchi, Model R110 Rotavapor; Switzerland) and 1 mL was removed for determination of radioactivity by liquid scintillation counting as above. The remaining extract was dried down to 2 mL as described above then stored at -7°C until HPLC-RD analysis. The extracted soils were air dried and stored at room temperature prior to analysis of non-extractable residues by combustion and counting. No downward movement of radioactivity was detected, and thus the data from the three core sections is pooled for presentation (data not shown).

2.5. Analytical methods

Radioactivity was determined by Liquid Scintillation Counting (LSC) using a Model LA 6500 instrument (Beckman Coulter, Irvine, CA). Each sample was added to 10 mL UniverSol scintillation cocktail (ICN, Costa Mesa, CA) in a plastic scintillation vial. Data was corrected automatically for quenching.

Parent compounds and potential transformation products in extracts were resolved by high performance liquid chromatography (Waters, Mississauga, ON) with UV and radioactivity detection (EG&G Berthold LB509 Radioflow Detector, Berthold GMBH & Co. KG., Bad Wildbad, Germany). A Zorbax Eclipse XDB C-18 column, (4.6 mm \times 250 mm, 5 μm pore size; Agilent, Santa Clara, CA) was used, and the UV detector (Varian 9050 Variable Wavelength UV/Vis Detector; Varian, Palo Alto, CA) was set at 274 nm for TCS and TCC, 210 nm for CBZ, and 238 for NAP. For TCS analyses, the mobile phase consisted of methanol:water in an 80:20 ratio. When delivered at 0.8 mL min^{-1} the retention time was 17.4 min for TCS, and 31 min for MeTCS. The same method was used to analyze TCC; retention times were 12.4 min for TCC, 4.3 min for potential transformation products 3,4-dichloroaniline and 3.8 min for 3-chloroaniline. Carbamazepine was analyzed using a mobile phase consisting of acetonitrile:water in

an 80:20 ratio. When delivered at 1 mL min^{-1} the retention time was 19.5 min for CBZ, and 11.8 min for the potential transformation product carbamazepine 10,11-epoxide. For naproxen analyses, the mobile phase consisted of acetonitrile:10 mM ammonium acetate in a 30:70 ratio. When delivered at 1 mL min^{-1} the retention time was 11.9 min.

Radioactivity in solid matrices was determined following combustion. One gram portions of material were mixed with 5 mg of cellulose powder, and the sample combusted at 900°C with a Harvey Oxidizer Model OX-500 (R.J. Harvey Instrument Corp; Tappan, NY). The evolved $^{14}\text{CO}_2$ was trapped with 15 mL ^{14}C -cocktail (Harvey) and the radioactivity was determined by LSC.

2.6. Data analyses for dissipation experiments

Dissipation rates for parent compounds were estimated on the basis of removal of total radioactivity from the extractable phase. The distribution of radioactive residues in extractable parent compounds and in transformation products was established on the basis of HPLC-RD retention time. Non-extractable soil-bound residues were determined by combusting extracted soil and quantifying the recovered radioactivity.

Raw data analysis was conducted using Microsoft Excel 2002 (Microsoft Canada, Toronto, ON). HPLC-RD data was captured and evaluated using Empower2 software (Waters, Mississauga, ON). Dissipation curves were plotted using SigmaPlot (Version 10, Systat Software Inc., Chicago, IL). Data in the figures represent the mean and standard deviation of triplicate samples. Statistically significant differences between treatments were established by T-tests (SigmaPlot). Rate constants and the number of days required to dissipate 50% of added residues (DT_{50}) were estimated using SigmaPlot polynomial curve fit procedure ($y = y_0 + ax$) and the Marquardt–Levenberg algorithm. Significant treatment effects on dissipation rate constants were evaluated using the Student's T test (Mullins, 2003) at 95% confidence and 4° of freedom. Rates were considered to be significantly different if the calculated T-value was greater than the critical T-distribution value of 2.78.

3. Results

3.1. Dissipation of triclosan

Extractable residues of ^{14}C -TCS were removed more rapidly from DMB placed below the soil surface ($DT_{50} = 17.3$ days) than when placed on the soil surface ($DT_{50} = 80.0$ days; Fig. 2, top panel). The decline in extractable residues approximated first order in both the surface treatment ($r^2 = 0.88$) and the sub-surface treatment ($r^2 = 0.96$; Table 2). Following 120 days of incubation, 7.1 ± 0.5 and $41.4 \pm 2.1\%$ of the initial ^{14}C -TCS remained in the extractable phase in the subsurface and surface-treatments, respectively (Fig. 2). Soil extracts were subjected to HPLC-RD analysis to determine what portion of the extractable radioactivity was in the form of parent compound, or any transformation product. In every case all detectable radioactivity recovered co-migrated with a TCS standard (data not shown). Following extraction, soils were combusted and counted to determine the non-extractable remaining radioactivity (Fig. 2, middle panel). Non-extractable residues were formed with a generally poor fit to a first order kinetic model, and without significant treatment effect (Table 2). Following 120 days of incubation, $28.6 \pm 6.4\%$ of the initially applied ^{14}C -TCS in the subsurface treatment remained in the non-extractable phase, whereas $23.8 \pm 5.1\%$ of the initial applied amount of ^{14}C -TCS in the surface treatment was likewise non-extractable. The atmospheric loss of ^{14}C -TCS residues at each sampling point was estimated by difference, based on the remaining extractable and non-extractable residues (Fig. 2; bottom panel). In the subsurface treatment, residues were lost atmospherically following a 7-day lag, and accounted for up to $77.2 \pm 1.5\%$ of the initial applied TCS after 60-days of incubation. In the surface treatment, residues were lost atmospherically only following a 40-day lag and

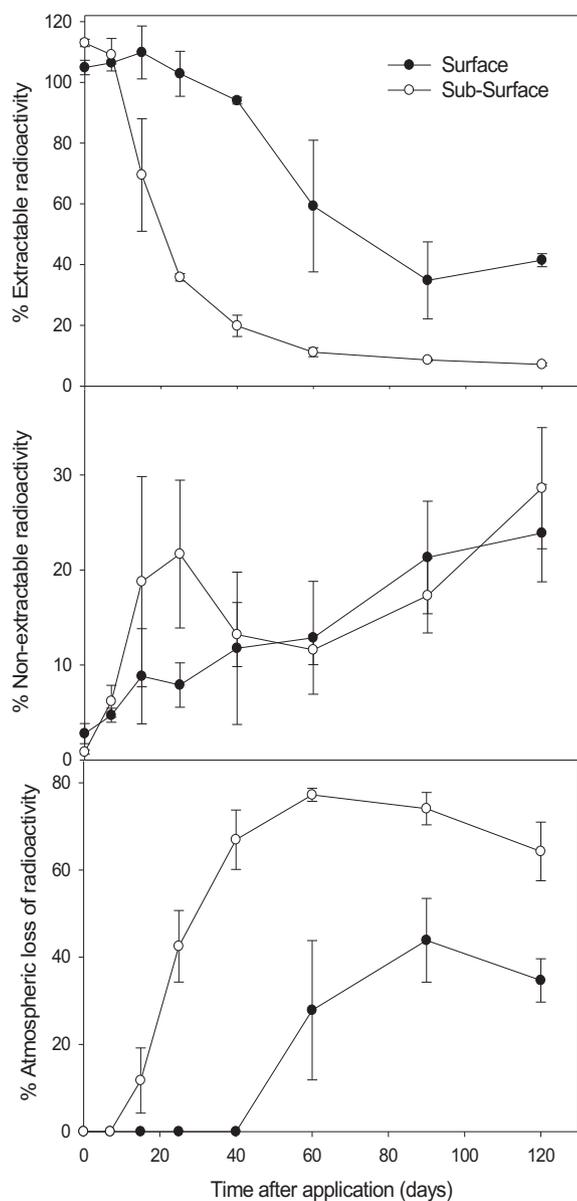


Fig. 2. Extractable residues (top panel), non-extractable soil residues (middle panel) and estimated atmospheric loss (bottom panel) of ^{14}C -triclosan in sandy loam soil cores incubated under ambient conditions. Triclosan was added to the soil in an aggregate of dewatered municipal biosolids (DMB) that was placed either on the soil surface or in the soil sub-surface.

Table 2

Fit of dissipation rate for each to a first order kinetic model. Indicated for each chemical is the fit to the model (r^2), rate constant (K), time to dissipate 50% of the initial concentration (DT_{50} ; day), and significance of treatment effect on the dissipation rate.

Chemical	Treatment	r^2	K (day^{-1})	Std. error	DT_{50} (day)
Triclosan	Surface	0.8804	0.0095 ^a	0.0018	72.9
	Sub-surface	0.9610	0.0400 ^b	0.0057	17.3
Triclocarban	Surface	0.7783	0.0044 ^a	0.0010	157.5
	Sub-surface	0.9201	0.0086 ^b	0.0012	80.6
Carbamazepine	Surface	0.9152	0.0071 ^a	0.0010	97.6
	Sub-surface	0.9841	0.0093 ^a	0.0006	74.5
Naproxen	Surface	0.9422	0.0674 ^a	0.0128	10.3
	Sub-surface	0.9915	0.2521 ^b	0.0206	2.7

^{a, b}: The superscript letter following the rate constants indicate that parameter is significantly different from the other treatments using a Student T test.

accounted for up to $34.7 \pm 5\%$ of the initially applied radioactivity after 92 days of incubation.

3.2. Dissipation of triclocarban

Extractable residues of ^{14}C -TCC were removed significantly faster ($DT_{50} = 80.6$ days; Table 2) following subsurface application than surface application ($DT_{50} = 157.5$ days; Fig. 3, top panel) of DMB. The difference in dissipation rate corresponded to a more rapid rate of non-extractable residue formation with the subsurface than surface-applied material. Following 120 days of incubation, 64.1 ± 14.5 and $32.8 \pm 4.1\%$ of the initial applied radioactivity remained extractable in the surface and the sub-surface treatments, respectively (Fig. 3). Soil extracts were subjected to HPLC-RD analysis to determine what portion of the extractable radioactivity was in the form of parent compound, or any transformation product. All the extracted radioactivity co-migrated with a TCC standard (data not shown). Extracted soils were

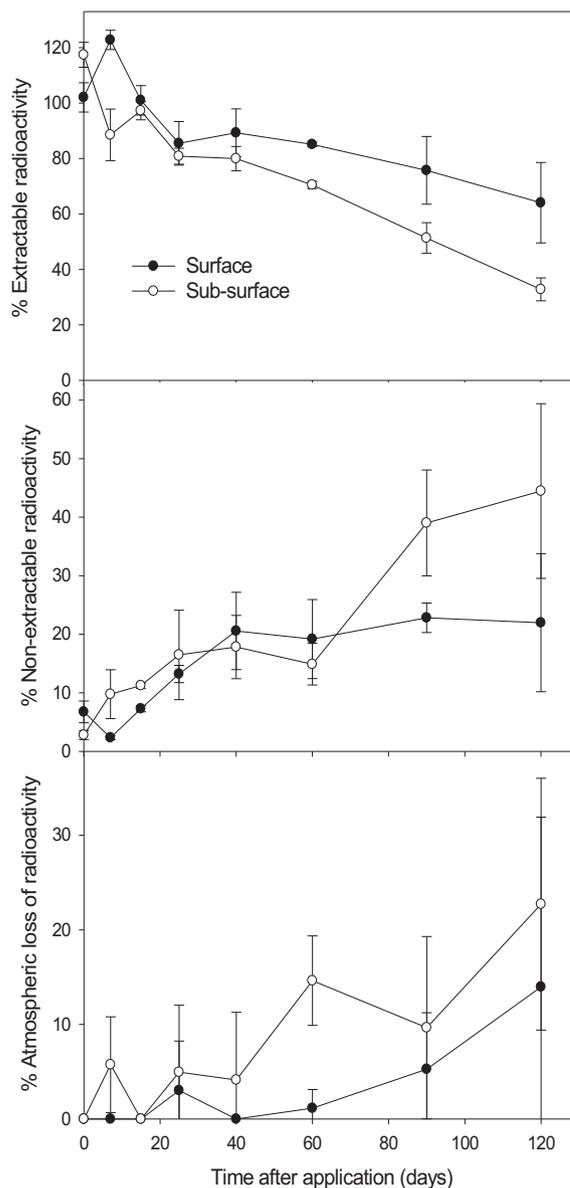


Fig. 3. Extractable residues (top panel), non-extractable soil residues (middle panel) and estimated atmospheric loss (bottom panel) of ^{14}C -triclocarban in sandy loam soil cores incubated under ambient conditions. Triclocarban was added to the soil in an aggregate of dewatered municipal biosolids (DMBs) that was placed either on the soil surface or in the soil sub-surface.

combusted and counted to determine the remaining non-extractable radioactivity (Fig. 2; middle panel). The formation of non-extractable residues was similar for both treatments until 60 days of incubation, reaching 19.2 ± 6.7 and $14.9 \pm 3.6\%$ of the initial applied amount of TCC for the surface and sub-surface treatments, respectively (Fig. 3). Thereafter, the formation of non-extractable residues in the sub-surface treatment was significantly faster than in the surface treatment until the end of incubation. Following 120 days of incubation 44.5 ± 14.9 and $22 \pm 11.8\%$ of the initial radioactivity was in the non-extractable phase in the subsurface and the surface treatments, respectively. The estimated atmospheric loss of TCC in the sub-surface treatment was higher than the surface treatment (Fig. 3, bottom panel). Following 120 days of incubation, an estimated 22.7 ± 13.3 and $14 \pm 17.9\%$ of the initial applied amount of TCC was lost atmospherically in the sub-surface and surface treatments, respectively.

3.3. Dissipation of carbamazepine

Residues of ^{14}C -CBZ were removed at comparable rates in the sub-surface ($\text{DT}_{50} = 74.5$ days) and surface ($\text{DT}_{50} = 97.6$ days) treatments (Table 2; Fig. 4, top panel). Following 120 days of incubation, 38 ± 9.4 and $32.3 \pm 5.4\%$ of the initial applied ^{14}C -CBZ remained extractable in the surface and sub-surface treatments, respectively (Fig. 4). Soil extracts were subjected to HPLC-RD analysis, and all the detectable radioactivity co-migrated with a standard of CBZ (data not shown). Non-extractable residues were formed with a generally poor fit to a first order kinetic model, and without significant treatment effect (Table 2; Fig. 4, middle panel). Following 120 days of incubation, non-extractable residues comprised about 20% of the initially applied radioactivity in both treatments (Fig. 4). In the subsurface treatment, atmospheric loss was linear after a lag period of about 15 days, whereas in the surface application treatment, the lag was 40 days. Following 120 days of incubation, atmospheric loss was estimated to represent

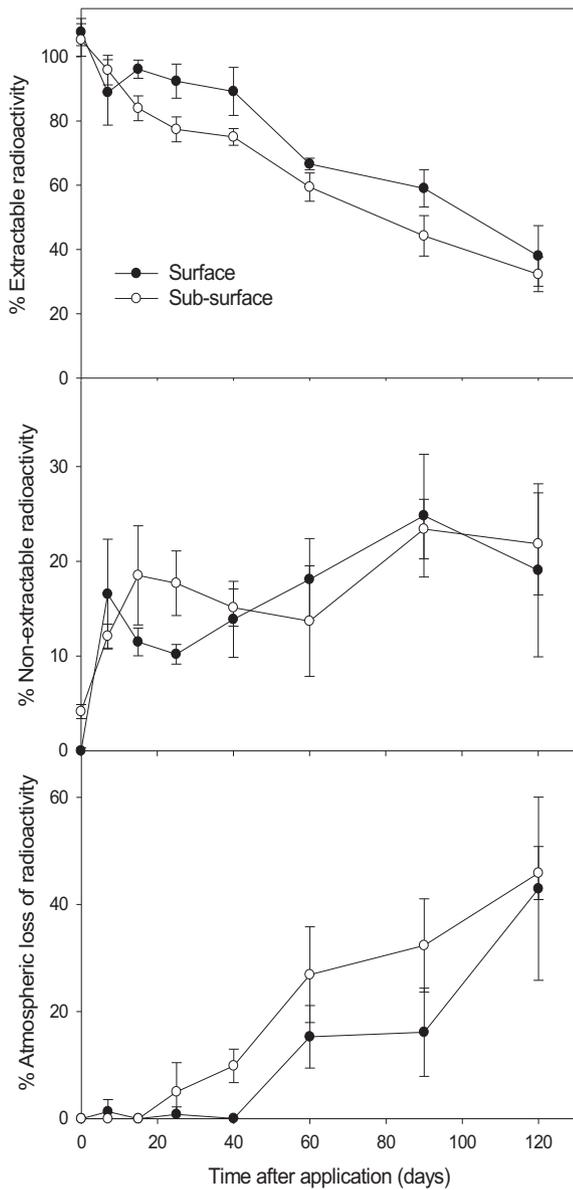


Fig. 4. Extractable residues (top panel), non-extractable soil residues (middle panel) and estimated atmospheric loss (bottom panel) of ^{14}C -carbamazepine in sandy loam soil cores incubated under ambient conditions. Carbamazepine was added to the soil in an aggregate of dewatered municipal biosolids (DMBs) that was placed either on the soil surface or in the soil sub-surface.

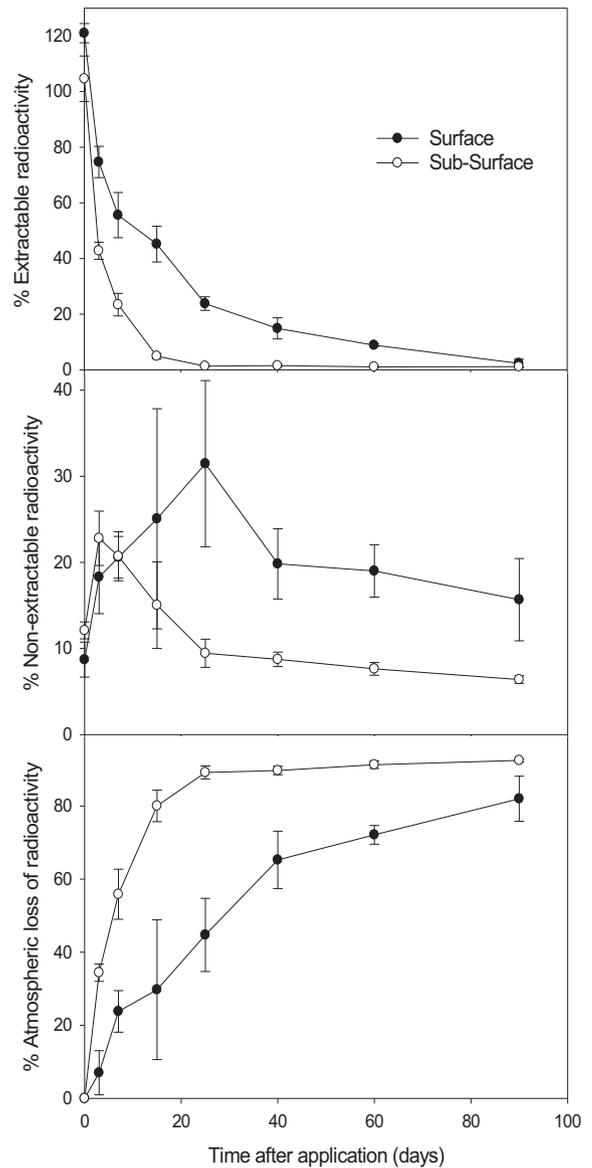


Fig. 5. Extractable residues (top panel), non-extractable soil residues (middle panel) and estimated atmospheric loss (bottom panel) of ^{14}C -naproxen in sandy loam soil cores incubated under ambient conditions. Naproxen was added to the soil in an aggregate of dewatered municipal biosolids (DMBs) that was placed either on the soil surface or in the soil sub-surface.

45.9 ± 4.9 and 42.9 ± 17.1% of the initially applied radioactivity in the sub-surface and surface treatments, respectively.

3.4. Dissipation of naproxen

Extractable naproxen residues were removed without a lag (Fig. 5). Extractable residues of ^{14}C -NAP declined more rapidly in the sub-surface treatment ($\text{DT}_{50} = 2.7$ days) than in the surface treatment ($\text{DT}_{50} = 10.3$ days; Table 2; Fig. 4, top panel). Following 90 days of incubation, the remaining extractable fraction in both treatments were comparable, representing about 2% of the initially applied radioactivity. Representative soil extracts were subjected to HPLC-RD analysis and 100% of the detectable radioactivity co-migrated with a standard of NAP (data not shown). Non-extractable residues were formed with unusual biphasic kinetics (Fig. 5, middle panel). In the sub-surface treatment, the non-extractable fraction reached a maximum of 23% of the initial radioactivity after day 3, declining thereafter. In the surface treatment, non-extractable residues increased steadily through day 26 to a maximum of about 32%, and declined thereafter. There was no lag for the atmospheric loss of ^{14}C -NAP in either treatment (Fig. 5, bottom panel). The rate of estimated atmospheric loss in the sub-surface treatment was more rapid in the sub-surface than the surface treatment, reaching a maximum of 92.5 ± 0.4 and 82 ± 6.5% of initially applied radioactivity, respectively, by the end of experiment.

3.5. Decay of surface-applied DMB aggregates

The decomposition of surface applied aggregates during the experimental period was determined gravimetrically (Fig. 6). Mass loss was slow and generally zero order (fit to a linear regression line has an r^2 of 0.8890), with about 80% of the dry mass initially applied still remaining after 120 days. There was no obvious association between precipitation events or variation in temperature and aggregate decomposition during the period of observation (Fig. 1).

4. Discussion

The dissipation of TCS, TCC and NAP was more rapid when biosolids aggregates were placed within the soil profile than when placed on the soil surface. The more rapid dissipation of TCS and NAP was associated with more significant atmospheric loss of radioactivity. It is assumed that the volatile radioactivity is $^{14}\text{CO}_2$ produced by mineralization of the ^{14}C atoms carried in the test substances. However, some possible loss of other volatile transformation products, for example methyl-TCS, cannot be discounted (Waria et al., 2011). With TCS, there was a markedly shorter lag phase and a larger portion of radioactivity lost to

the atmosphere in the sub-surface applied material compared to surface applied. With NAP, the rate of atmospheric loss was faster and final proportion lost larger with sub-surface applied material compared to surface applied. In contrast, the more rapid dissipation of TCC with sub-surface applied material was associated with more significant formation of non-extractable residues in the latter part of the incubation, and not with a difference in apparent atmospheric loss of radioactivity. Furthermore, non-extractable TCC- ^{14}C were formed more rapidly from sub-surface applied material following a lag of 60 days, suggesting that it was TCC transformation products rather than the parent compound that were preferentially fixed. These significant apparent atmospheric loss of TCC ^{14}C residues are in contrast with those from a laboratory study that found negligible mineralization of ^{14}C -TCC when spiked into anaerobically digested biosolids and incorporated into fine sand or silt loam soils (Snyder et al., 2010). Taken together, results from the present study indicate that for TCS, TCC and NAP the depth of placement significantly affected both the kinetics of removal of parent, and the mass balance of transformation products as determined by the disposition of ^{14}C .

In contrast to TCS, TCC and NAP, CBZ dissipation was independent of the depth of placement. This result is consistent with greater recalcitrance of CBZ to biodegradation. Carbamazepine was reported to be dissipated only very slowly in three different soils with the half-life ranging from 46 to 173 days (Li et al., 2013). In this study, over a 120-day laboratory incubation with only negligible amounts of ^{14}C -CBZ were mineralized or formed non-extractable residues, recovery of ^{14}C in the form of extractable parent and various transformation products was almost stoichiometric. Perhaps the much higher fraction of non-extractable residues in the present study was due to the chemical being carried in biosolids rather than delivered directly to the soil, or the outdoor incubation conditions.

The discordance between the mass loss of DMB aggregates on the soil surface (Fig. 6) and the kinetics of dissipation of ^{14}C -residues in surface-applied aggregates indicates that the physical decomposition of aggregates was not rate-limiting for dissipation of the test compounds. Following 120 days of incubation aggregate mass had decreased by about 20%, whereas reduction in pharmaceutical mass ranged from 100% (NAP) to about 35% (TCC). Dissipation processes occurring in the aggregate, volatilization, and leaching of ^{14}C -residues into the underlying soil followed by dissipation are processes that could be contributing to dissipation of chemicals carried in surface applied biosolids. The mineralization of naproxen in soil was accelerated by liquid municipal biosolids, and these yield enrichment cultures that converted naproxen to the corresponding naphthol, O-desmethyl naproxen (Topp et al., 2008a). Triclosan added to activated sludge was converted to a series of products indicating that it was subject to methylation, sulfation, hydroxylation and ether cleavage reactions (Chen et al., 2015). Activated sludge contains microorganisms that mineralized TCC radiolabeled in either ring (Gledhill, 1975). Carbamazepine was removed very slowly by nitrifying enrichment cultures (Dawas-Massalha et al., 2014), but was completely stable in nitrifying activated sludge (Kruglova et al., 2014). In both the surface and subsurface-applied aggregates a very significant portion of the [carbonyl- ^{14}C]-CBZ was lost to the atmosphere (Fig. 4). The fungi *Trametes versicolor* for example is capable of removing the carboxamide moiety of carbamazepine to form acridine and acridone (Jelic et al., 2012).

It should be noted that soil dissipation studies using residues spiked into biosolids may underrepresent the persistence of 'native' chemical residues carried in biosolids (Langdon et al., 2013). Residues that have persisted through wastewater treatment, sorbed to the organic phase and persisted during biosolids preparation are likely to be far less bio-available and more persistent than freshly added. Nevertheless, the soil dissipation rates reported in the present study fall within the range reported from a variety of other studies (Table 3).

In practice, dewatered biosolids can range in size and consistency from a friable powder, to fist-sized clods, to a viscous paste. The

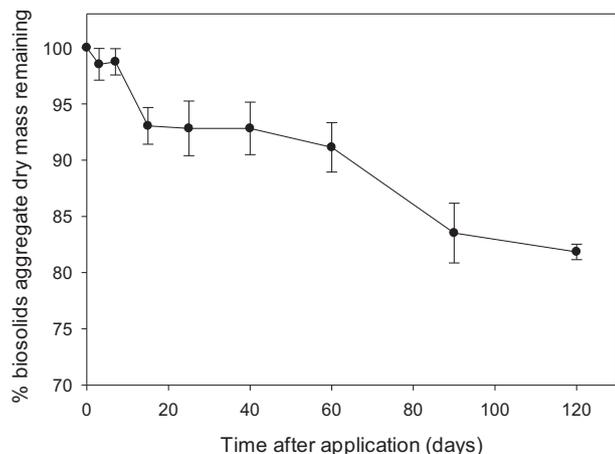


Fig. 6. Decomposition and mass loss of surface-applied DMB aggregates during the course of the experiment.

Table 3Some representative reported rates (time to dissipate 50% of the residues; DT₅₀) for soil dissipation of the investigated compounds.

Chemical	Experimental conditions	DT ₅₀ (days)	Reference
Triclosan	Field study in South Australia	No marked decrease in concentration	Langdon et al. (2012)
	Field study, Northern Virginia, USA	107.4	Lozano et al. (2010)
	Incubation in controlled conditions	16.4	Tsekoura et al. (2011)
	Outdoor mesocosms, Maryland, USA	182–193	Walters et al. (2010)
	Field study in north China	106–258	Chen et al. (2014b)
Triclocarban	Field study in north China	133	Chen et al. (2014a)
	Field study in China	51–191	Chen et al. (2014b)
Carbamazepine	Incubation in controlled conditions	>120	Snyder et al. (2010)
	Incubation in controlled conditions	355–1,624	Dalkmann et al. (2014)
Naproxen	Outdoor mesocosms, Maryland, USA	462–533	Walters et al. (2010)
	Incubation in controlled conditions	6–19	Dalkmann et al. (2014)
	Incubation in controlled conditions	3.9–15.1	Monteiro and Boxall (2009)
	Incubation in controlled conditions	17.4–69.3	Lin and Gan (2011)

dimensions of dewatered biosolids aggregates vary widely according to a number of factors including the treatment processes used on the sewage sludge at recovery (eg. flocculation agents used, belt or centrifuge dewatering), and subsequently (eg. digestion, heat treatment). Likewise critical is the equipment used to apply the material (eg. extrusion, beater type spreader), and the force used at discharge. The method and rigor of soil incorporation following surface application will determine how thoroughly biosolids clods are broken up and how exposed they are to atmospheric processes like rain and radiation, and how much contact they have to the soil matrix. Mouldboard plowing as a means to incorporate biosolids in a field can reduce aggregate occurrence on the soil surface (Gottschall et al., 2012). Also, equipment that directly injects dewatered biosolids beneath the soil surface (eg. Terratec Environmental Ltd.'s dewatered biosolids direct injection system) deposits an extrudate with a diameter of several (eg. 5) centimeters, specified by the dimensions of the exit tube (Edwards et al., 2009). All of these considerations will impact the texture and aggregate dimensions of biosolids, their degree of soil contact, and constraints for gas and solute diffusion. Overall, results reported in the present study will likely not be generalizable to instances where the biosolids have dimensions very different from the one experimented with here.

Taken together, results from the present study indicate that pharmaceuticals amenable to microbial transformation are dissipated more rapidly when biosolids are deposited in the soil profile than when left on the soil surface. Sub-surface placement will increase the availability of biosolids-borne contaminants to the soil microflora, as well as attenuate fluctuations in moisture and temperature that could inhibit microorganisms carried in the biosolids that are able to decompose the chemicals. This result is consistent with a similar effect of crop residue placement on residue decomposition rate (Helgason et al., 2014). The more rapid dissipation of ¹⁴C-residues in surface applied biosolids than can be explained by physical decomposition of the aggregates is clear evidence that biodegradation takes place within the aggregates. However, soil incorporation is clearly preferable with respect to accelerating the dissipation of chemical constituents in biosolid aggregates, reducing the physical availability of residues on the soil and therefore risk of surface runoff, and reducing vector attraction and odor emissions following application (Edwards et al., 2009).

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