



Fate of pharmaceuticals and pesticides in fly larvae composting



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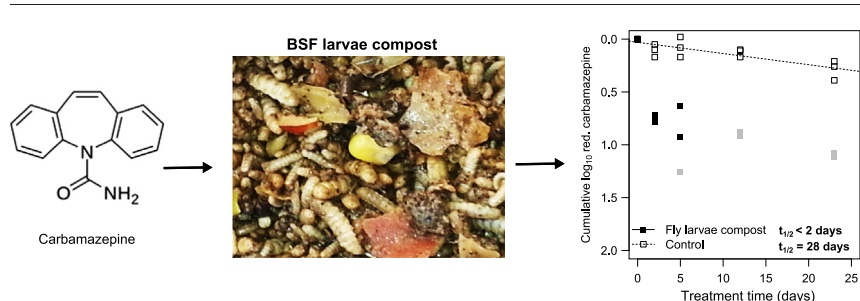
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HIGHLIGHTS

- Degradation of pharmaceuticals and pesticides in fly larvae composting (FLC).
- Half-life considerably shorter in FLC than in control with no larvae.
- Half-life of carbamazepine was less than two days in FLC.
- No bioaccumulation in larvae detected.
- FLC could impede the spreading of pharmaceuticals and pesticide in the environment.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel and efficient organic waste management strategy currently gaining great attention is fly larvae composting. High resource recovery efficiency can be achieved in this closed-looped system, but pharmaceuticals and pesticides in waste could potentially accumulate in every loop of the treatment system and spread to the environment. This study evaluated the fate of three pharmaceuticals (carbamazepine, roxithromycin, trimethoprim) and two pesticides (azoxystrobin, propiconazole) in a fly larvae composting system and in a control treatment with no larvae. It was found that the half-life of all five substances was shorter in the fly larvae compost (<10% of control) and no bioaccumulation was detected in the larvae. Fly larvae composting could thus impede the spread of pharmaceuticals and pesticides into the environment.

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1. Introduction

Inadequate or absent organic waste management can result in severe negative environmental and anthropogenic impacts. Pathogens present in organic wastes, e.g. *Salmonella* spp., can contribute to the spread of diseases (Hoorweg and Bhada-Tata, 2012), while the nutrients in organic waste can cause eutrophication if leached into water

bodies. However, if applied to arable land, these nutrients can instead be of value, e.g. in the production of agricultural crops (Diacono and Montemurro, 2010). Capturing the nutrients found in organic wastes could greatly alleviate the environmental burden, by avoiding the negative impact of inadequate treatment and reducing the need for chemical fertilisers (Good and Beatty, 2011). However, the cost of collection and treatment of the organic fraction in the waste is not covered by the value of the products, which often consist of compost and/or biogas (Diener et al., 2011b). Thus unless organic waste management is subsidised, use of the waste even for production of biogas is not

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economically viable (Gebrezgabher et al., 2010). Another pressing issue globally is the increased consumption of livestock products, which has led to ever greater demand for animal feed (Steinfeld et al., 2006). The recent high demand for protein feedstuffs has encouraged many unsustainable practices, such as soy bean production on virgin land and overfishing of wild fish stocks (Alder et al., 2008; Elizabeth et al., 2010). In recent years, fly larvae composting has gained considerable attention as an alternative organic waste management strategy (Čičková et al., 2015). In this composting technology, fly larvae, e.g. of the black soldier fly (BSF, *Hermetia illucens*) convert organic waste into two valuable products: animal feed protein in the form of fly larvae (Stamer, 2015) and a treatment residue that can be used as organic fertiliser (Kováčik et al., 2010). The larvae of BSF are high in protein (40%) and fat (30%) (St-Hilaire et al., 2007). Fly larvae composting is an efficient and quick composting method for organic waste, with degradation rates of up to 70% on a dry matter (dm) basis being demonstrated (Diener et al., 2011a). The greatest advantage of fly larvae composting from a waste management perspective is the generation of valuable products, which brings about a shift in the organic waste value chain, enabling the treatment to bear its own costs (Diener, 2010).

The hazards associated with release of toxic substances (e.g. pesticides) in the environment have been known for many decades and have resulted in the banning of many chemical agents and strict measures to avoid unnecessary spreading. It is well known that if toxic pollutants enter surface water bodies, there is a risk of these or their metabolites reaching end-points where they can cause adverse effects on ecological systems (Köhler and Triebkorn, 2013). However, environmental pollution by pharmaceuticals only started to be recognised as a serious concern during the past decade. The pharmaceutical industry is constantly developing new therapeutic agents, many of which are persistent, resulting in biologically active pharmaceuticals being found intact in the aquatic environment and posing a risk of unintentional exposure in humans and wildlife (Brodin et al., 2013; Khetan and Collins, 2007). In closed-loop systems, such as fly larvae composting systems, the fate of pharmaceuticals and pesticides is of even greater interest, as these substances could potentially accumulate in every loop of the treatment system. In a previous study, Zhang et al. (2014) examined removal of common antibiotics in a combined fly larvae (*Musca domestica*)/vermicomposting system and observed cumulative removal rates of around 70% for oxytetracycline, chlortetracycline and sulfadiazine, while the reduction in other antibiotics investigated was 30–40%. However, up-to-date knowledge on the fate of pharmaceuticals and pesticides in fly larvae composting systems is limited.

The objectives of this study were thus to assess: i) potential bioaccumulation of selected pharmaceuticals and pesticides in BSF larvae, and ii) potential degradation of pharmaceutical and pesticide residues over time in fly larvae composting compared with a control with no larvae. Five biologically active compounds were selected based on their common use and persistence in the environment: the pharmaceuticals carbamazepine, roxithromycin and trimethoprim and the pesticides azoxystrobin and propiconazole.

2. Materials and methods

2.1. Target compounds

The target compounds were: carbamazepine (CAS 298-46-4), roxithromycin (CAS 80214-83-1), trimethoprim (CAS 738-70-5), azoxystrobin (CAS 131860-33-8) and propiconazole (CAS 60207-90-1). Carbamazepine (carboxamide-¹³C, ¹⁵N) and trimethoprim-d₉ were used as mass-labelled internal standards (IS).

2.2. Experimental set-up

Dog food (Purina Pro Plan puppy; 40% dm) was used as the feed (compost) substrate, as it is comparable to food waste in terms of

nutrient composition and fat/energy content (Vinnerås et al., 2003). At the start of the experiment, 63 g of substrate were inoculated with the selected pharmaceuticals (carbamazepine 1.8–1.9 mg g⁻¹ dm, trimethoprim 5.9–9.9 mg g⁻¹ dm, roxithromycin 5.8–5.9 mg g⁻¹ dm) and pesticides (azoxystrobin 2.4–4.6 mg g⁻¹ dm, propiconazole 3.2–14.1 mg g⁻¹ dm). Bioaccumulation in the larvae and degradation in the substrate were investigated in batch systems over a period of 27 days in three different set-ups comprising: i) spiked substrate and BSF larvae (spiked BSF; n = 3), ii) spiked substrate and no BSF larvae (control substrate; n = 3) and iii) unspiked substrate and BSF larvae (control BSF; n = 1) (Fig. 1).

The spiked BSF treatments were fed with uncontaminated substrate on days 2, 5, 7, 9, 12, 14 and 16, at a feeding rate of 100 mg substrate larva⁻¹ day⁻¹ (Diener et al., 2009). Similar amounts were added to the control treatments, to replicate the dilution effect on the concentration of the target substances. The uncontaminated substrate was mixed into the treatments in the control, while it was added without mixing to the larvae treatments as the movement of the larvae was assumed to be sufficient.

2.3. Sampling

Each larval treatment received 450 10-day-old larvae (average weight 14 mg larvae⁻¹) at the start of the experiment. On days 2 and 3, 50 larvae were removed and on day 5 and 12, 35 larvae were removed for analyses of pharmaceuticals and pesticides. The remaining larvae were allowed to develop into prepupae, and in total 165–230 prepupae were collected between day 21 and day 27 as they migrated from the feed. The remaining prepupae were allowed to develop into flies and were collected between day 64 and day 104 as flies. Upon collection, larvae, prepupae and flies were allowed to empty their gut for 24 h and were then stored at -19 °C until analysis. A set of 10 g samples of substrate (treatment residues) were collected from the larvae treatment and control on days 2, 5, 12 and 23 and stored at -19 °C until analysis.

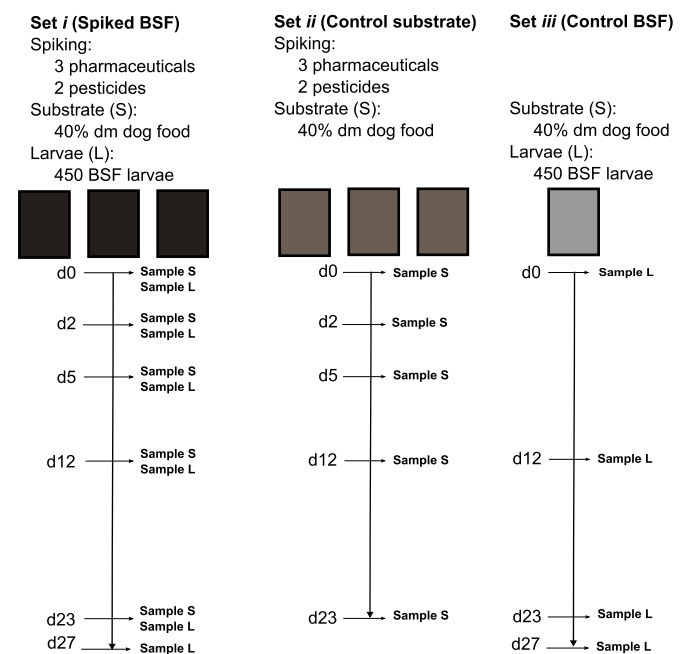


Fig. 1. Schematic representation of the experimental set-up comprising: i) spiked BSF treatment, ii) spiked substrate control and iii) unspiked control BSF; and the timeline of sampling of substrate (Sample S) and of larvae (Sample L) on days 0, 2, 5, 12, 23 and 27.

2.4. Analyses

For the analyses, 0.5 g portions of freeze-dried sample (*i.e.* larvae, prepupae, flies, food substrate) were placed in tubes, methanol (5 mL) was added and each sample was spiked with 100 μL of the IS mixture (conc. 0.25 $\text{ng } \mu\text{L}^{-1}$). The samples were vortexed for 1 min, sonicated for 15 min and then centrifuged at 3500 rpm for 15 min. This extraction process was repeated twice more and the three methanol extracts were pooled (in total 15 mL extract). The extracts were frozen for 1 h ($-20\text{ }^\circ\text{C}$) to allow the fat to precipitate and then filtered through 0.2 μm regenerated cellulose syringe filters (Scantec Nordic). Following this, the extracts were diluted to 250 mL with HPLC grade water and purified by solid phase extraction (SPE) using Oasis HLB cartridges (Waters, 200 mg, 6 mL). For the SPE clean-up, the cartridges were conditioned using 6 mL methanol followed by 6 mL HPLC grade water and then loaded with the sample at a flow rate of approximately 1 mL min^{-1} . After sample loading, the cartridges were washed with 6 mL HPLC grade water and then dried under vacuum for 30 min. The target compounds were eluted with 4×2 mL aliquots of methanol. Extracts were evaporated under nitrogen stream to dryness and then reconstituted with 1 mL methanol. The final concentrations of constituents were measured by ultra-high-performance-liquid chromatography (UHPLC, Waters Corporation, USA) coupled to quadrupole-time of flight mass spectrometry (QTOF Xevo G2S, Waters Corporation, Manchester, UK). Chromatographic separation was achieved in 11 min using an Acquity HSS T3 column (1.8 μm particle size, 2.1×100 mm) at a flow rate of 0.5 mL min^{-1} . Mobile phases used were a) HPLC grade water with 0.1% formic acid and b) acetonitrile with 0.1% formic acid. The column temperature was set at $40\text{ }^\circ\text{C}$, the sample manager temperature at $15\text{ }^\circ\text{C}$ and the injection volume was 5 μL . Samples were analysed by positive electrospray ionisation, using MS^E experiments in the resolution mode. In this type of experiment, two acquisition functions with different collision energies are created: a low energy (LE) function with a collision energy of 4 eV and a high energy (HE) function with a collision energy ramp ranging from 10 to 45 eV. The MS data in the LE function were acquired over a m/z range of 100–1200 in a scan time of 0.25 s. Calibration of the mass axis from m/z 100 to 1200 was conducted daily with a 0.5 mM sodium formate solution prepared in 90:10 (v/v) 2-propanol/water. For automated accurate mass measurement the lock spray probe was employed, using a lock mass leucine encephalin solution (2 $\text{ng } \mu\text{L}^{-1}$) in ACN/water (50/50) with 0.1% formic acid. Data were evaluated using the operating software UNIFI™ (Waters Corporation). Target pharmaceuticals and pesticides were positively identified in the samples using the following criteria: a) accurate mass measurements of the precursor ion $[\text{M} + \text{H}]^+$ in the LE function, with an error below 5 ppm, b) the presence of at least one characteristic m/z ion in the HE function, and c) the UHPLC retention time of the compound compared with that of a standard ($\pm 2\%$).

2.5. Calculations

2.5.1. Reduction by dilution

The expected concentration of the substance in the substrate at $t(i)$, assuming no decay, was calculated as:

$$C_{t(i)} = C_{t(0)} \times \frac{\text{dm.tot}_{t(0)}}{\text{dm.tot}_{t(i)}} \quad (1)$$

where $C_{t(0)}$ is the initial concentration, the $\text{dm.tot}_{t(0)}$ and $\text{dm.tot}_{t(i)}$ is the total amount of dry matter at time 0 and i , respectively.

2.5.2. Half-life

The half-life ($t_{1/2}$) was calculated using the equation for first-order kinetics as:

$$y_t = y_0 \times 10^{-kt} \quad (2)$$

where $y(t)$ is the substance concentration at time t , y_0 is the initial substance concentration, t is the time and k is the rate constant. At $t_{1/2}$, the concentration has dropped to half and is thus calculated as:

$$t_{1/2} = \frac{\log_{10}(2)}{k} \quad (3)$$

2.6. Statistics

Linear regression models with 95% confidence interval were conducted to establish significance levels ($p < 0.05$) and rate constants for the substance degradation. All analyses were performed, and graphical representations produced, in R version 2.0.2 (R Core Team, 2012).

3. Results and discussion

3.1. Background data on the target substances

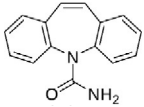
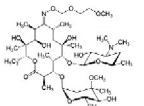
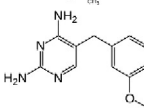
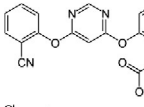
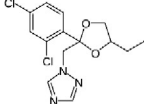
A summary of the properties of the selected substances reported in the literature is presented in Table 1. Carbamazepine is a common antiepileptic and antipsychotic pharmaceutical that can be found in water bodies connected to sewage treatment plants all around the world, as it is generally not removed during wastewater treatment (Garcia et al., 2012). Furthermore, it has been found to be highly persistent and is e.g. not degraded during anaerobic digestion (Carballa et al., 2007). Roxithromycin is a broadly used antibiotic that is persistent in aquatic environments and is not degraded under aerobic conditions (which usually prevail in wastewater treatment plants) (Kolpin et al., 2002; Liu et al., 2014). However, Carballa et al. (2007) found 94% degradation of roxithromycin during anaerobic digestion. Trimethoprim is a common antibiotic often used to treat urine infections. High concentrations of trimethoprim have been detected in wastewater effluents (Kolpin et al., 2002), indicating low removal rate in sewage treatment processes. Even with UV radiation, the highest degradation rate of trimethoprim achieved is 60% (Kim et al., 2015). Azoxystrobin is a broad-spectrum fungicide, used mainly for wheat and barley in the Nordic countries, which has been shown to display large variations in degradation rate in soil, with half-life values ranging from 3 to 279 days (Lewis et al., 2015b). Jørgensen et al. (2012) demonstrated azoxystrobin leaching from agricultural sites for a long time after application and, since it is highly toxic, this could pose a risk to aquatic organisms. Propiconazole is a foliar fungicide (Lewis et al., 2015b) that has been demonstrated to be stable in soils, with a half-life of over one year in silty-clay soils (Kim et al., 2003).

3.2. Development of larvae and change in substrate over time

The larvae gained weight at rates reported previously for BSF larvae fed on high energy substrates (Banks et al., 2014; Diener et al., 2011a) (Table 2). However, the fat content on a dm basis in the prepupae (40%) was somewhat higher than previously reported (30%) in a study where the larvae were reared on poultry manure (St-Hilaire et al., 2007). Poultry manure has a fat content of around 6% on a dm basis (Ghaly and MacDonald, 2012), while the dog food used in our experiment had a fat content of around 16% on a dm basis. Caruso et al. (2014) reported a fat content of 38% on a dm basis in BSF larvae reared on palm kernel meal containing 9–15% fat on a dm basis. The spiked BSF larvae in the present study had a higher fat content than those in the control, which could potentially be related to the impact of the spiked substances. However, the reason for this increase and the underlying mechanism are not known.

The concentrations of the selected substances were investigated over time and the reduction relative to the initial amount was calculated. During the course of the experiment, unspiked food was added to the treatment in order to feed the larvae and was also added in the

Table 1
Physico-chemical characteristics of the target pharmaceuticals and pesticides.

Structure	Area of application	Chemical formula	Molar mass (g M ⁻¹)	Log K _{ow} ^a	Water solubility (mg L ⁻¹)	Half-life in soil (t _{1/2} , days)	BCF ^b	K _{oc} ^c
<i>Pharmaceutical</i> Carbamazepine 	Antiepileptic	C ₁₅ H ₁₂ N ₂ O	236.095	2.45 ^d	112 (25 °C) ^d	46.2 ^e	1.9 ^f	168.6 ^g
Roxithromycin 	Antibiotic	C ₄₁ H ₇₆ N ₂ O ₁₅	836.525	2.75 ^h	0.0189 (25 °C) ^h	≥ 120 ⁱ	13.8 ^j	7.21 ^g
Trimethoprim 	Antibiotic	C ₁₄ H ₁₈ N ₄ O ₃	290.138	0.91 ^k	400 (25 °C) ^k	110 ^k	4 ^h	78.6 ^g
<i>Pesticide</i> Azoxytrobilin 	Fungicide	C ₂₂ H ₁₇ N ₃ O ₅	403.117	2.56 ^l	6.7 (20 °C) ^l	78 ^l	21 ^m	589 ^l
Propiconazole 	Fungicide	C ₁₅ H ₁₇ Cl ₂ N ₃ O ₂	341.070	3.72 ^l	150 (20 °C) ^l	214 ^l	116 ^l	1086 ^l

^a Octanol–water partition coefficient.^b Bioconcentration factor.^c Soil organic carbon–water partition coefficient.^d US EPA (2016).^e Li et al. (2013) found that the decay rate in various soils varied greatly: fine sandy clay loam (46.2 days); loam (173.3 days); silty clay (>120 days).^f Garcia et al. (2012); for muscle tissue of *P. notatus*.^g High range 1680–3990 (Domènech et al., 2010; Sarmah et al., 2006).^h Chen et al. (2011).ⁱ Schlüsener and Bester (2006).^j Maximum BCF in *Carassius auratus* muscle tissue after exposure to 4 µg L⁻¹ roxithromycin (Liu et al., 2014).^k Lewis et al. (2015a).^l Lewis et al. (2015b).^m HSDB (2012).

control system. These additions of unspiked food led to dilution of the substances investigated (Eq. (1)). Before the first addition of unspiked food, a 60% ($\frac{m_{t1}}{m_{t0}} = 0.4 \pm 0.2$) reduction in total mass had occurred in the fly larvae compost, while the reduction in the control during the same period was just above 10% ($\frac{m_{t1}}{m_{t0}} = 0.9 \pm 0.02$) (Table 2). Until day 12 the increase in dry mass was larger in the control, but by the end of the experiment the mass accumulation was larger in the fly larvae compost, by a factor of 6.5 compared with a factor of 5.6 in the control. This is most likely because the larvae had stopped feeding (the last addition of substrate occurred on day 16 and the first prepupae migrated

on day 21), since during the moulting process and prepupal stage feeding ceases (Tomberlin et al., 2002).

3.2.1. Pharmaceuticals and pesticides in larvae

The selected substances were not detected in the control larvae (detection limit <0.1 ng g⁻¹ wet weight, ww). In the larvae in the spiked substrate, only one of the added compounds was detected on a few occasions in one of the treatments, the antibiotic trimethoprim, with 24 and 6 ng g⁻¹ ww on day 3 and day 12, respectively (on day 12, <1% of the spiked concentration on a ww basis). The high fat content of the larvae made extraction challenging, and it is possible that substances

Table 2
Summary of larval development (weight and fat content (% dm)) and change in total initial substrate dm ($\frac{m_{t1}}{m_{t0}}$) in the spiked BSF treatment, the control substrate and the control BSF over the course of the experiment, reported as mean ± standard deviation.

	Day 0	Day 2	Day 5	Day 12	Day 23	Day 27
Larvae weight (mg):						
Spiked BSF (n = 3)	14	54 ± 8.0	89 ± 22	227 ± 16	189 ± 27 ^a	225 ± 28 ^a
Control BSF (n = 1)	14	63	137	263	186 ^a	214 ^a
Larvae fat content (% dm):						
Spiked BSF (n = 3)	28.1	NA ^b	NA ^b	25 ± 3.0	42.9 ± 0.2 ^a	47.0 ± 7.5 ^a
Control BSF (n = 1)	28.1	NA ^b	NA ^b	26	41 ^a	40 ^a
Change from total initial substrate dm ($\frac{m_{t1}}{m_{t0}}$):						
Spiked BSF (n = 3)	1.0	0.4 ± 0.2 ^c	1.8 ± 0.2	4.2 ± 0.2	6.5 ± 0.5	
Control substrate (n = 3)	1.0	0.9 ± 0.04 ^c	3.1 ± 0.1	5.9 ± 0.1	5.6 ± 0.2	

^a Analysed on migrated prepupae.^b NA = not available.^c Sampled on day 3.

present in the larvae were not detected. No other pharmaceutical or pesticide was detected in the larvae at any point using the extraction methods described above.

3.2.2. Degradation of pharmaceuticals in the substrate

The concentration of all pharmaceuticals analysed was reduced over time in both the spiked BSF substrate and the control substrate (Fig. 2), and the reduction appeared to follow first-order kinetics (Eq. (2)). The reduction by dilution was greater in the control than in the spiked BSF (Fig. 2a, c and e). This is to be expected, as more substrate accumulated in the larval biomass in the spiked BSF. The cumulative reduction in total amount of carbamazepine and roxithromycin added was close to 1 log unit after 3–5 days in the spiked BSF treatment, while it was just over 0.5 log unit for trimethoprim (Fig. 2b, d and f). In the control, the greatest cumulative reduction was observed for roxithromycin, with close to 1 log unit reduction at the end of the experiment (Fig. 2d).

The reduction for carbamazepine and trimethoprim was <0.5 log unit even after 23 days (Fig. 2b and f).

For pesticides, the difference between the spiked BSF and the control was less pronounced (Fig. 3). For both azoxystrobin and propiconazole, cumulative reductions of around 1 and 0.5 log unit were achieved in the spiked BSF, while a reduction of close to 1 log unit was seen in the control (Fig. 3b and d). The cumulative reduction in propiconazole at day 5 was around 1.5 log units, while at day 12 it was only around 1 log unit. It should be noted that on days 5 and 12 the concentration in one out of three replicates was below the detection limit. It is not clear why the measured concentration was more or less the same on day 5 and day 12 even though the amount of substrate had increased (from a factor of 1.8 on day 5 to a factor of 4.2 on day 12), which should have diluted the concentration. It is possible that this discrepancy was caused by insufficiently homogenised substrate during sampling, or by measurement uncertainties due to quantification just above the limit of detection (LOD). After day 12, no propiconazole was detected in the

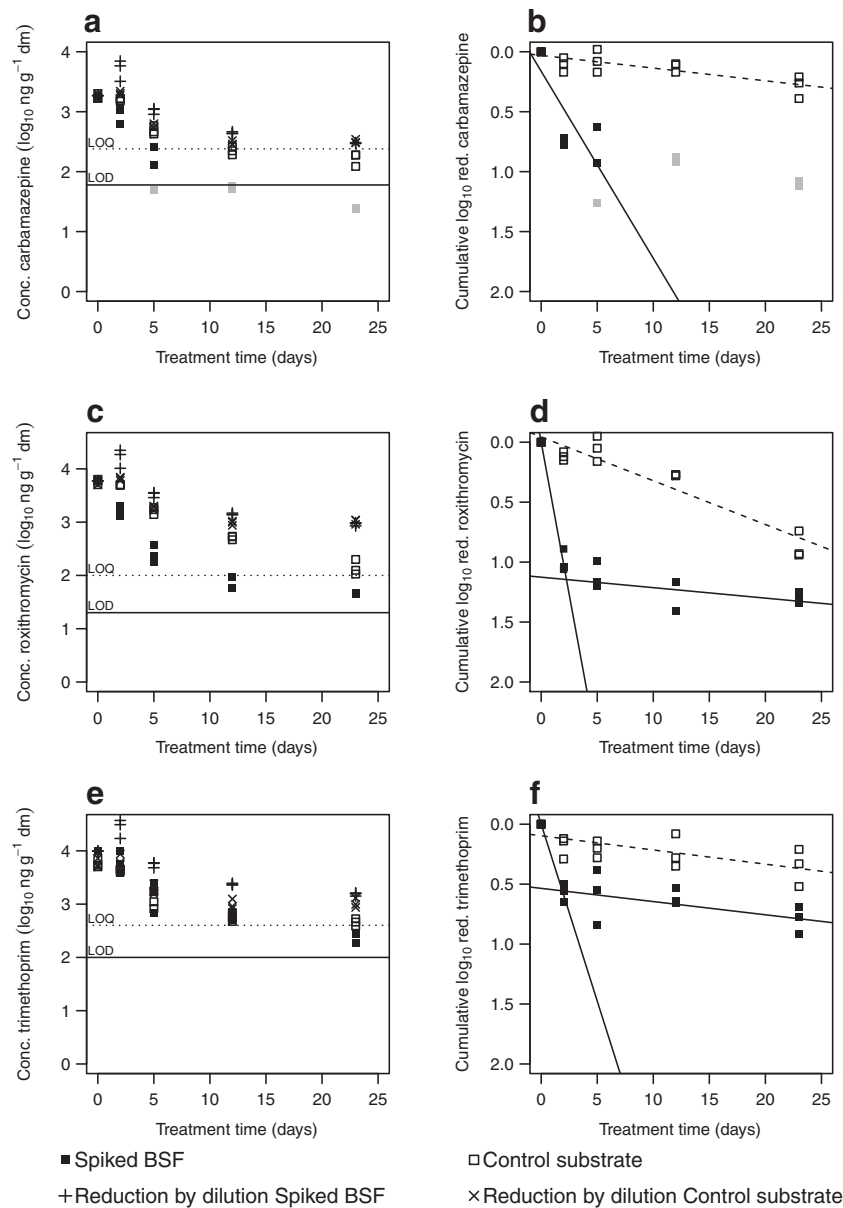


Fig. 2. Reduction in different pharmaceuticals in substrate in the spiked BSF treatment (■) and control substrate (□). a, c) and e): \log_{10} concentration ($\log_{10} \text{ ng g}^{-1} \text{ dm}$) over time, including the concentration reduction by dilution in the spiked BSF (+) and control substrate (×) and limit of detection (LOD —) and limit of quantification (LOQ---), for carbamazepine, roxithromycin and trimethoprim. b, d) and f): cumulative \log_{10} reduction in total initial mass in the system of the three pharmaceuticals over time and regression line for reduction over time in the spiked BSF (—) and the control substrate (---). Note: values below the LOD are shown in grey (■).

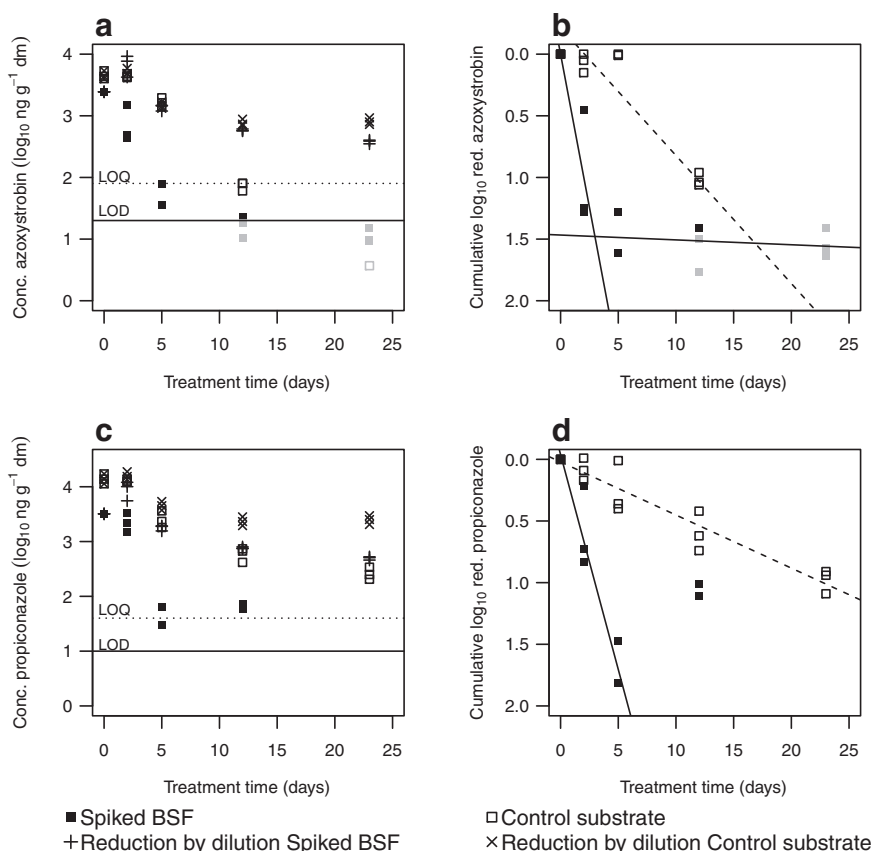


Fig. 3. Reduction in different pesticides in the substrate in the spiked BSF treatment (■) and the control substrate (□). a) and c): \log_{10} concentration ($\log_{10} \text{ ng g}^{-1} \text{ dm}$) over time, including the concentration reduction by dilution in the spiked BSF (+) and control substrate (x) and limit of detection (LOD —) and limit of quantification (LOQ---) for azoxystrobin and propiconazole. b) and d): cumulative \log_{10} reduction in total initial mass in the system of two pesticides over time and regression line for reduction over time in the spiked BSF (—) and in control substrate (---). Note: values below the LOD are shown in grey (■).

substrate (detection limit $10 \text{ ng g}^{-1} \text{ dm}$). It is thus likely that the points on day 12 are outliers, and they were therefore not taken into consideration in the half-life time calculations.

The cumulative reduction in the spiked BSF treatment followed first-order kinetics in two steps, associated with two different rate constants, k_1 and k_2 , while the decay rate in the control substrate was only associated with one rate constant, k (Table 3). The half-life of the substances was determined using k_1 for the spiked BSF treatment and k for the control substrate (Eq. (3)). In general, a larger difference in half-life was observed for the pharmaceuticals than for the pesticides in both the treatment and the control, and the half-life was shorter for the pesticides than for the pharmaceuticals. Among the pharmaceuticals, the smallest difference between treatment and the control was found for roxithromycin, even though the half-life was shorter (0.6 days) in the spiked BSF than in the control (8.2 days). There was a considerable difference in half-life for both carbamazepine and trimethoprim (<2 days in the spiked BSF, compared with 25 and 26 days, respectively, in the control). For all substances, the half-life was shorter in the spiked BSF. Furthermore, all substances were reduced to a higher extent in the spiked BSF over the course of the experiment except the pesticide azoxystrobin, which was reduced to a greater extent in the control substrate.

The rate constant for the first step was statistically significant for all substances ($p < 0.05$), but the rate constant for step 2 in the spiked BSF was not significant ($p > 0.05$), suggesting that little or no decay occurred after the first few days of larval composting. Additional substrate was added for the first time on day 5 and thus the reduction in concentration of the selected substances could be merely due to dilution, as no significant cumulative reduction was observed after day 2 or day 5 (Table 3). The difference in half-life observed between the spiked BSF and the control strongly indicates that the significant and fast reduction in the

target substances in the fly larvae compost was due to larval activity. Since no substance was detected in the larval biomass, it can be assumed that the substances were degraded in the substrate. Most of observed degradation occurred prior to addition of unspiked substrate, which diluted the substances in the remaining substrate. This suggests that no more larva-associated decay was possible once the concentration fell below a certain level (i.e. $50 \text{ ng g}^{-1} \text{ ww}$ for carbamazepine; $2500 \text{ ng g}^{-1} \text{ ww}$ for trimethoprim; $650 \text{ ng g}^{-1} \text{ ww}$ for roxithromycin, $340 \text{ ng g}^{-1} \text{ ww}$ for azoxystrobin and $580 \text{ ng g}^{-1} \text{ ww}$ for propiconazole). It should be noted that the accuracy of analysis decreases just above the LOD, which results in increased uncertainty of estimated cumulative reduction.

Trimethoprim was the substance that was reduced least (<1 log unit) in the BSF composting treatment. It has a relatively low K_{ow} and has higher water solubility than the other substances investigated here (Table 1). It is possible that the high fat content (16% on dm basis) of the substrate facilitated uptake of the other substances by the larvae, while trimethoprim that partitioned to the water phase to a greater extent, was less available for uptake.

It is likely that degradation of the substances was associated with microbiological activity. Li et al. (2013) found no degradation of carbamazepine in sterilised soil, but a half-life close to 50 days in fine (unsterilised) sandy clay loam soil and concluded that soil microflora plays an important role in degradation of carbamazepine. In the present study, the experiment was conducted with dog food as substrate at 25°C under aerobic conditions, favouring microbial activity. In the spiked BSF compost, the half-life of carbamazepine was <2 days, an exceptionally small value for this compound (Li et al., 2013; Monteiro and Boxall, 2009). In BSF composting, the larvae are known to condition the material with their own gut microflora, facilitating feed intake by the

Table 3

Rate constants k_1 , k and k_2 and correlation (R^2 values) of the substance decay equation and half-life time ($t_{1/2}$), calculated assuming first-order kinetics (Eq. (2)) and k_1 values, of the pharmaceuticals and pesticides in the spiked BSF and the control substrate, and the cumulative reduction (\log_{10}) by day 23.

	k_1/k	R^2	$t_{1/2}$ (days)	k_2	R^2	Cumulative reduction (\log_{10}) by day 23 (mean \pm sd, $n = 3$)
Carbamazepine						
Spiked BSF	0.156***	0.65	1.9			1.1 \pm 0.02 ^a
Control substrate	0.0163*	0.66	28			0.3 \pm 0.08
Roxithromycin						
Spiked BSF	0.498***	0.99	0.6	0.0097 [†]	0.38	1.3 \pm 0.04
Control substrate	0.0365***	0.90	8.2			0.9 \pm 0.09
Trimethoprim						
Spiked BSF	0.285***	0.98	1.1	0.0118 [†]	0.31	0.8 \pm 0.1
Control substrate	0.0118**	0.47	25			0.4 \pm 0.1
Azoxystrobin						
Spiked BSF	0.497*	0.77	0.6	0.0039 [†]	0.04	1.5 \pm 0.1
Control substrate	0.10379***	0.91	2.9			2.4 ^{a,c}
Propiconazole						
Spiked BSF	0.34860***	0.93	0.9			>1.7 ^b
Control substrate	0.043064***	0.91	7.0			1.0 \pm 0.08

^a Value below LOD.

^b No value measured, estimated as LOD.

^c Based on one value.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

larvae (Yu et al., 2011). This BSF-associated microflora may have facilitated degradation of the target substances investigated here, which could explain the difference in decay rates in the spiked BSF and control substrate.

Based on the results found in this study, there is no great risk of substance accumulation in the closed-loop BSF composting system. Furthermore, the fly larvae composting system investigated in this study was not optimised in terms of feeding rate, retention time and larval density; higher reductions of these compounds may thus be possible in a more optimised system.

4. Conclusions

The half-life of all five substances investigated was shorter in the spiked BSF treatment than in the control with no larvae. The half-life in the spiked BSF treatment and control with no larvae was, respectively: 1.9 days and 28 days for carbamazepine; 0.6 days and 8.2 days for roxithromycin; 1.1 days and 25 days for trimethoprim; 0.6 days and 2.9 days for azoxystrobin; and 0.9 days and 7 days for propiconazole. The total cumulative reduction by day 23 in the BSF treatment and control was, respectively: 1.1 and 0.3 \log_{10} unit for carbamazepine; 1.3 and 0.9 \log_{10} unit for roxithromycin; 0.8 and 0.4 \log_{10} unit for trimethoprim; 1.5 and 2.4 \log_{10} unit for azoxystrobin; and 1.7 and 1 \log_{10} unit for propiconazole. No bioaccumulation in the larvae was detected for any of the target substances. BSF composting of different organic waste fractions could thus reduce the risk of spread of pharmaceuticals and pesticides in the environment when applying composted material to arable land.

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