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Degradation of diclofenac and triclosan residues in sewage sludge compost

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Abstract. Land application of sewage sludge compost is an important and efficient tool in the remediation of industrial landscapes and agricultural soils in Estonia. A number of studies have shown that, as a rule, pharmaceuticals and personal care products (PPCPs) are neither completely removed by sewage treatment, nor completely degraded in the environment. In this study, degradation rates of diclofenac sodium (DFC) and triclosan (TCS) were determined during sewage sludge composting. Anaerobically digested and dewatered sewage sludge was mixed with sawdust at two different ratios (1:2 and 1:3 sludge/sawdust, v:v). Then aerobic composting was carried out. These ratios were chosen on the basis of previous studies on sewage sludge composting with different bulking agents. The initial concentration of DFC and TCS was 2 mg kg^{-1} in relation to dry weight (dw). Low quantities of the studied pharmaceuticals were present in sewage sludge that was used for preparing the compost mixtures used in our experiments. The background concentrations of DFC and TCS were never equal to zero. The results showed that the difference between sewage sludge and bulking agent ratios (1:2 vs 1:3) in compost samples did not significantly affect temperature profiles during the experiment. The degradation of pharmaceuticals was more complete in the compost samples where the ratio of bulking agent was higher (1:3 by volume). The average degradation level (in all compost mixtures) was 95% for DFC and 68% for TCS. Pharmaceuticals entering into the soil may affect microbial activity, plant growth and development, and may have adverse effects on living organisms.

Key words: sewage sludge compost, sawdust, fertilizers, diclofenac, triclosan.

INTRODUCTION

Compost has proven to be a valuable matter in land recultivation and forestry (Haiba et al., 2016; Järvis et al., 2016). Estonia has the world's largest exploited oil-shale basin covering about 4% of its territory. In 2001−2013 the number of active landfills in Estonia decreased from 159 to 13. Recultivation of the landscapes covered by semi-coke, oil-shale ash mountains, abandoned opencast mines and closed landfills appears to be one of the major environmental tasks in Estonia (Haiba et al., 2016). The formation of soil with its typical biota is crucial for the restoration of former mining areas and remediation of waste heaps (Kalda et al., 2015). Compost based on sewage sludge could be a useful tool in overcoming the problems associated with land recultivation. Sewage

sludge contains useful organic matter and nutrients for plants (Kaonga et al., 2010). The contents of nitrogen, phosphorus and organic matter are up to 10 times higher in sewage sludge and its compost, if compared to common Estonian agricultural soils.

Composting is the major way of making the soil application of sewage sludge safer. Still, its usage as a fertilizer is limited due to a large number of toxic pollutants found in this matter (Lillenberg et al., 2010). In particular, the presence of pharmaceutical residues, even in very low concentrations. in sewage sludge compost is of great concern. The widespread use of antibiotics is the most important factor for the emergence, selection, and dissemination of antibiotic-resistant bacteria (Baquero et al., 2008; Roasto et al., 2009; Munir et al., 2011; Naquin et al., 2015; Mäesaar et al., 2016). Due to the occurrence of antibiotic resistance genes in the wastewater treatment systems, the impact of the antibiotic combinations is greater than the sum of their independent activities (Aydin et al., 2015). As a result the bacteria may develop several resistance mechanisms; this will ultimately result in multidrug resistance (Baharoglu & Mazel, 2011).

Recent years have shown intensive work directed to the development of reliable methods for the determination of pharmaceutical residues in the environment (Lillenberg et al., 2009; Kipper et al., 2011; Garcia-Rodriguez et al., 2014; Casado et al., 2015; Morales-Toledo et al., 2016), showing the increasing importance of this phenomenon. Pharmaceuticals can be degraded during composting (Poulsen & Bester, 2010; Kim et al., 2012). Among the factors which possibly promote micropollutants degradation during composting is the presence of fungi in the composted matter (Zhang et al., 2011). However, the literature data on this topic are scarce and more research is required in this area (Butkovskyi et al., 2016).

Diclofenac (DFC) is one of the most popular non-prescription medications. It is non-steroidal anti-inflammatory drug and widely used for relieving pain (Chen et al., 2015). DCF together with its human metabolites enter wastewater treatment plants (WWTPs) through sewers (Zhang et al., 2008; Sagristà et al., 2010). This is one of the most frequently detected drugs in WWTPs, having low removal efficiency and often found in high concentrations in effluent water (Stülten et al., 2008; Al-Rajab et al., 2010; Bartha et al., 2014; Osorio et al., 2014). DFC residues have been detected in sewage sludge with concentrations reported from 2 ng g^{-1} to 140 ng g^{-1} (Jelić et al., 2009; Dobor et al., 2010; Jelić et al., 2011; Loos et al., 2013). DCF residues have been detected in aqueous environment (Al-Rajab et al., 2010) where they can cause DNA damage with induced immunosuppression and genotoxicity in fish (Ribas et al., 2014). Chemical structure of DCF involves a chlorine atom and therefore its residues are not readily biodegradable in the environment. Metabolism of DFC has been studied and described in mammals, fungi and microorganisms (Huber et al., 2012; Bartha et al., 2014). DFC is acutely toxic to birds and presumably could leach into soil beneath the corpses of livestock containing DFC residues (Stülten et el., 2008; Al-Rajab et al., 2010).

Triclosan (TSC) is a broad-spectrum antimicrobial compound, commonly used in personal care products (soaps, creams, toothpastes, detergents) and housewares (cutting boards, even textiles and toys). This compound has been used for over 40 years. The use of antimicrobials and -bacterial products is increasing all over the world (Lozano et al., 2010). Today, TSC compounds are consumed in Europe at approximately 350 tons per year (Pintado-Herrera et al., 2014). TSC residues have been detected in wastewater (in concentrations ranging from 1–10 μgL^{-1}) as well as in sewage sludge (concentration range 2–8 mg kg^{-1} dry matter) (Chen et al., 2011; Loos et al., 2013). TCS residues have

been found in soil fertilized with sewage sludge compost up to a concentration of 4 μg kg^{-1}. Various studies have shown that already at relatively low concentration TSC may have adverse effects to the environment – prevents bacterial metabolism, affects microbial respiratory activity and populations (Lozano et al., 2010; Chen, et al., 2011; Pintado-Herrera et al., 2014).

Though a variety of compounds and their metabolites are present in the environment, their biodegradation and ecotoxicological effects are not well known (Li et al., 2014). Toxic compounds and pharmaceutical residues in soil can affect microbial activity, plant growth and development and may have adverse effects on living organisms (Lillenberg et al., 2010). Accumulation of antimicrobials from soil into foodplants may pose a danger, as very small amounts of these drugs in everyday food may generate the strains of resistant bacteria in humans (Kipper et al., 2010).

Sawdust has proven to be an efficient bulking agent for sewage sludge composting (Banegas et al., 2007). The purpose of this pilot study was to determine the impact of different proportions of bulking agent (sawdust) on the degradation of DFC and TCS residues in sewage sludge compost.

MATERIALS AND METHODS

Chemicals and materials

Standard substances of pharmaceuticals were obtained from Sigma-Aldrich: diclofenac sodium salt (99.9%) and triclosan (99.7%). As liquid chromatography – mass spectrometry (LC-MS) eluent components, methanol (≥ 99.9%; LC-MS Ultra CHROMASOLV; Fluka), water purified in-house using Millipore Milli-Q Advantage A10 system, 1,1,1,3,3,3-hexafluoroisopropanol (HFIP, Sigma-Aldrich), NH_4OH (25%; eluent additive for LC-MS; Fluka) and formic acid (≥ 98%; puriss p.a., Sigma-Aldrich) were utilized. For sample preparation, vortex mixer VWR International, shaker Elpan 358S, centrifuge Eppendorf 5430R and ultrasonic bath Bandelin Sonorex were used. Sample extracts were filtered through Sartorius Minisart RC4 (regenerated cellulose, pore size 0.2 μm, membrane diameter 4 mm) syringe filters using disposable 2 ml syringes (Brand).

Sample collection

The anaerobically digested and dewatered by centrifugation sewage sludge samples were collected from municipal wastewater treatment plant in Tallinn (440,000 inhabitants), Estonia. The sewage sludge was mixed with sawdust at two different ratios (1:2 and 1:3 sludge: sawdust, v:v) and submitted to a process of aerobic composting. These ratios were chosen on the basis of literature (Banegas et al., 2007) and our previous studies on sludge composting with different bulking agents (straw, sawdust, oil-shale ash, wood chips) (Haiba et al., 2013; Nei et al., 2014; Nei et al., 2015). The initial concentration of every pharmaceutical was 2 mg kg^{-1} in relation to dry weight (dw). In addition to this, two reference piles (without additions of pharmaceuticals) were prepared. The content of compost samples is presented in Table 1.

Table 1. Compost samples

Sample No	Compost mixture	Mixture ratio (v:v)	Dry matter*, %	Added pharmaceuticals in compost sample
K1	Sewage sludge: sawdust	1:2	35.3	2 mg kg^{-1} (dw)
K2	Sewage sludge: sawdust	1:2	35.2	Not added
K3	Sewage sludge: sawdust	1:3	40.3	2 mg kg^{-1} (dw)
K4	Sewage sludge: sawdust	1:3	40.8	Not added

* – dry matter in the beginning of experiment.

Sample preparation

Samples were thawed at room temperature and mixed by vigorous shaking. For extraction, about 5 g of sample was precisely weighted into 50 ml polypropylene centrifuge tube. The following extraction procedure was used:

1. 15 ml of extraction solvent (1% v/v formic acid in ethanol) was added to a sample tube.
2. The mixture was Vortex-mixed for 30 s.
3. The sample tube was tightly capped and placed horizontally on a shaker (200 rpm) for 10 min.
4. The tube was turned into vertical position and shaken by hand to ensure that the solid contents are in contact with extraction solvent.
5. Extraction was continued by sonicating for 10 min.
6. Samples were centrifuged at 7,830 rpm for 5 min.
7. The extract was removed from the tube using pipette.

Extraction steps 1–7 were repeated five times with each sample. Extracts were combined in 100 ml polypropylene bottles, mixed and weighted. From each extract 15 ml was taken into 15 ml polypropylene centrifuge tube for further treatment.

Prior to LC-MS/MS analysis, sample extracts were diluted: to 100 µl extract 1,400 µl of MilliQ water were added in 1.5 ml Eppendorf tube. Automatic pipette was used for dosing, but all the solutions were weighted. The solutions were vortex-mixed and filtered through syringe filter. First five drops of filtrate were discarded and the remaining (ca 1 ml) was collected into auto-sampler vial (2 ml glass vial).

Calibration and quality control samples

Calibration and quality control samples were prepared by diluting stock solutions of analytes. Stock solutions were prepared by dissolving appropriate amount of analytes in methanol. Working standards were prepared in 1.5 ml Eppendorf tubes by diluting 600 µl of stock solution with 400 µl MilliQ water. Similarly to preparation of sample solutions, all solutions were prepared by weight, vortex-mixed and filtered through syringe filters. Concentration of calibration and quality control solutions were chosen according to the linear range for each analyte.

LC-MS/MS analysis

Sample extracts were analyzed using LC-MS/MS system consisting of ultra-high performance liquid chromatograph UHPLC Agilent 1290 Infinity and mass spectrometer Agilent 6495 Triple Quad. The liquid chromatograph consisted of the following modules: binary high-pressure gradient pump with built-in degasser, autosampler with sample compartment cooling and column thermostat. Waters XBridge C18 (150 mm ×

3 mm, 3.5 μm) analytical column and Waters Guard Cartridge (20 mm × 4.6 mm) (Waters) precolumn were used for sample analysis.

For analyte detection triple quadrupole mass spectrometer equipped with heated electrospray interface (HESI) Agilent JetStream was used. Chromatographic separation was carried out using gradient elution. As the weak component of eluent (A), 5 mM HFIP buffer solution (pH adjusted to 9 using NH_4OH) was used. The strong component of the eluent (B) was methanol. The gradient program started from 10% B and content of B was increased to 100% during 33 minutes. For the following 3 minutes isocratic (100% B) elution was used, followed by 3 min gradient to 10% B. For equilibration the column was eluted with 10% B for 4 minutes. Eluent flow rate was 0.3 ml min^{-1}, column temperature maintained at 30 °C and injection volume 10 μl. Multiple reaction monitoring (MRM) mode was used for analyte detection. MRM transitions used are presented in Table 2.

Table 2. MRM transitions, collision energies (CE) and ionization polarities used for analysis

Analyte	Precursor ion, *m/z*	Product ion, *m/z*	CE	Polarity mode
Diclofenac	296	250	10	Positive
	296	214*	40	Positive
Triclosan	289	37*	20	Negative
	289	35	10	Negative
	287	35	15	Negative

* – quantitative transition.

The following ion source and MS parameters were used for analysis: drying gas temperature 250°C and flow rate 14 l min^{-1}, nebulizing gas pressure 20 psi (138 kPa), heating gas temperature 350 °C and flow rate 11 l min^{-1}, capillary voltage 3,000 V. As drying, nebulizing, heating and collision gas nitrogen was used. The instrument was controlled using Agilent MassHunter Workstation ver B.07.00 software. For quantitative analysis Agilent MassHunter Workstation Quantitative analysis ver B.07.01 software was used.

Composting

Experiments were performed in non-transparent plastic containers. With the aim of preventing heat loss from the sides and bottom of the containers, a 5 cm thick insulation (glass wool) was used. Compost samples of about 30 L were prepared with each mixture. The solutions of pharmaceuticals were prepared as follows: 2 mg of each pharmaceutical was dissolved in 100 ml ethanol and after that 400 ml distilled water was added to the solution. Then the solutions of the studied pharmaceuticals (DCF and TSC) were mixed with compost samples. The room temperature was 23–26 °C. Compost samples were turned periodically (5–6 times per week) to provide sufficient aeration and homogenization. The moisture content of the mixtures was maintained at 60–70% of their water holding capacity throughout the composting period. The temperature of each mixture was monitored daily at 3–4 different points in each sample with a digital temperature probe and mercury thermometer. The duration of experiment was 30 days. The samples were homogenized before analysing – taken randomly from different parts of the sample.

Determination of the microbial characteristics of sewage sludge compost

The methodology used for the determination of microbial characteristics of sewage sludge compost is presented in Nei et al. (2014). Soil microbial Substrate Induced Respiration rates (SIR) were measured using manometric respirometers (Oxitop®, WTW) (Platen & Wirtz, 1999). 50 g of fieldmoist compost was amended with glycose and incubated in a closed vessel at 22 °C in the dark for 24 hours. After incubation the microbial biomass C was calculated.

To determine the microbial to fungal ratio, selective inhibition technique was used. In order to assess the fungal biomass, samples were treated with streptomycin (12 g kg^{-1}) and glucose (5 g kg^{-1}); for the determination of bacterial biomass, samples were treated with cyxloheximide (6 g kg^{-1}) and glucose (5 g kg^{-1}). Reference samples were treated with cyxloheximide (12 g kg^{-1}) and streptomycin (6 g kg^{-1}). All the samples were incubated in closed vessels at 22 °C in the darkness for 24 hours and then the biomass C was calculated (Nei et al., 2014).

RESULTS AND DISCUSSION

In the beginning of the experiment the growth of microbial population caused the rise of temperature drastically in compost samples with pharmaceuticals (samples K1 and K3), if compared to reference samples (K2 and K4) (Fig. 1). Although SIR profiles seemed similar in the case of all four compost samples (Table 3), the highest temperatures in compost samples K1 (57.5 °C) and K3 (52.5 °C) differed from the temperature peaks in samples K2 (42.2 °C) and K4 (41.4 °C) more than 10 °C. The reason for that might have been the difference in the ratios between fungi and bacteria (Table 3). Compost samples with pharmaceuticals (K1 and K3) had a lower ratio of fungi and bacteria. The formation time of bacteria is much shorter than that of fungi. They are smaller and therefore abundant in compost (Chroni et al., 2009). Bacteria have a more active metabolism and due to this it was natural that in the beginning of the experiment the temperature rose faster in compost samples K1 and K3.

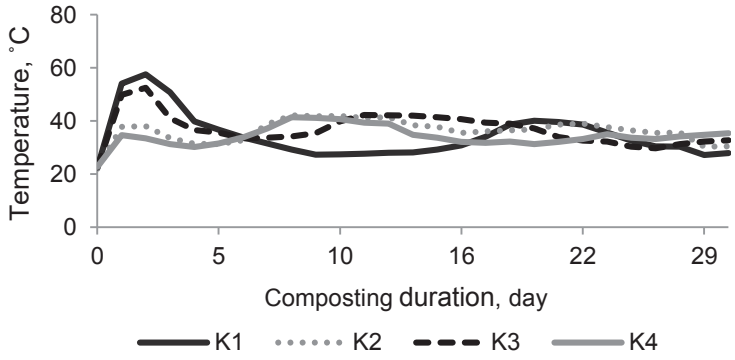

Figure 1. Temperature profiles during one month composting for 1:2 (v:v) sewage sludge – sawdust mixtures: K1 – containing pharmaceuticals; K2 – without pharmaceuticals and for 1:3 (v:v) sewage sludge – sawdust mixtures: K3 – containing pharmaceuticals; K4 – without pharmaceuticals.

After one week the ratio of fungi and bacteria was reduced in compost samples with additional pharmaceuticals, but biomass of microorganisms had increased in the case of samples K2 and K4. It could be the reason for higher temperatures in samples K2 and K4 (Fig. 1).

Table 3. The average bacterial-to-fungal ratio, substrate induced respiration (SIR) profiles and moisture content during 30 days

Sample No	Ratio of fungal to bacteria	SIR, mg biomass C g^{-1} dw	Moisture, %
K1	0.974 ± 0.072	13.8 ± 3.5	62.6 ± 0.4
K2	0.980 ± 0.075	19.9 ± 1.2	62.6 ± 0.3
K3	0.909 ± 0.062	17.3 ± 3.2	61.6 ± 0.4
K4	0.965 ± 0.065	16.2 ± 1.1	62.2 ± 0.4

The results of the analyses indicated that none of the compost samples was originally free of DCF and TSC residues (see Table 4). Although DFC concentrations were found in relatively low amounts, the concentrations of triclosan were up to 2 mg kg^{-1} (dw). A well-managed composting process resulting in an efficient decline of residual pharmaceuticals, as shown in Kim et al. (2012), requires some extra source of organic matter, as the organic matter can elevate temperatures and provide a wide range of additional binding sites during composting. Sawdust is an organic source able to initiate efficient composting, as exhibited by elevated composting temperatures. According to Kim et al. (2012), this consequently resulted in the reduction of residual concentrations of pharmaceuticals to acceptable levels in a relatively short composting period.

After adding the pharmaceuticals to the compost mixtures their initial concentrations in dry matter were determined again. All of the concentrations were above the expected values (see Table 4) probably due to the rapid adsorption of pharmaceuticals (from liquid phase) to solid particles of sewage sludge or bulking agent. This is in agreement with the data presented in previous publications (Golet et al., 2003; Göbel et al., 2005; Yang et al. 2011; Nei et al., 2014). After one week, the concentrations of the studied pharmaceuticals were determined again. The concentrations of DFC and TCS residues had decreased by 51% and 29% in compost mixtures with sludge-sawdust ratios 1:2 (v:v). In the case of compost samples with the ratios of 1:3 (v:v) the relevant concentration drops were 42% (DFC) and 28% (TCS).

Table 4. Concentrations of diclofenac and triclosan in sewage sludge – sawdust compost samples (mg kg^{-1}, dw)

Compound	Sample No	Before spiking	1 day	1 week	1 month
Diclofenac	K1	0.086 ± 0.004	2.646 ± 0.319	1.307 ± 0.035	0.209 ± 0.010
	K3	0.064 ± 0.005	2.381 ± 0.212	1.369 ± 0.044	0.036 ± 0.002
Triclosan	K1	**1.768 ± 0.062**	4.541 ± 0.378	3.241 ± 0.202	**2.068 ± 0.138**
	K3	**1.232 ± 0.070**	3.528 ± 0.143	2.538 ± 0.089	**0.682 ± 0.019**

According to the data presented in Table 5 it is evident that the degradation of pharmaceuticals was more complete when higher ratio of sawdust was used in preparing compost mixtures.

Table 5. Extent of degradation (%) for diclofenac and triclosan during one month composting

Sample No	Diclofenac	Triclosan
K1	92	55
K3	98	81
Average	95	68

These results show clearly, that the degradation of TCS takes place only partly during one-month composting period, indicating that longer periods are needed for the more complete removal of pharmaceutical residues from sewage sludge based compost.

CONCLUSIONS

The study was carried out to demonstrate the degradation of DCF and TCS in composting processes using different ratios of sewage sludge and bulking agent (sawdust). There is strong evidence that biotic and abiotic factors contributed to the decomposition of pharmaceuticals during composting. The selection of appropriate composting technologies is clearly important in the view of decreasing the levels of pollutants in compost to acceptable levels. Higher ratios of sawdust in the mixture with sewage sludge clearly speeded up the degradation of both DCF and TCS. The results showed that the difference between sewage sludge and bulking agent ratios (1:2 vs 1:3) in composts did not significantly affect temperature profiles during the experiment. The degradation of pharmaceuticals was more complete in the compost samples where the ratio of bulking agent was higher (1:3 by volume). 30-days composting period was not sufficient for degrading TCS residues present in sludge-sawdust mixtures, whereas almost full degradation (98%) of DCF took place in the case of 1:3 sludge-sawdust sample. It is an extremely complicated task to secure the removal of organic pollutants from sewage sludge compost. More research is needed to clarify the factors speeding up the degradation of different pharmaceuticals during composting. Special attention should be payed to the intelligent and safe application of such composts.

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