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On the degradation of metformin and carbamazepine residues in sewage sludge compost

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Abstract. Recent decades have shown intensive studies devoted to the fate of pharmaceuticals in the environment. These studies have involved the development of analytical tools, determination of pharmaceuticals in different compartments, composting technologies, and plant uptake of pharmaceuticals. The presence of organic pollutants in sewage sludge, including pharmaceuticals, is a problem of major concern. The re-use of sewage sludge should be encouraged since it represents a long-term solution provided that the quality of the sludge re-used is compatible with public health and environmental protection requirements. Composting is a widely recognized way of making the soil application of sewage sludge safer.
In this study, the impact of sewage sludge composting on the degradation of metformin (MET), by far the most often prescribed antidiabetic drug worldwide, and carbamazepine (CBZ), a poorly biodegradable but widely used as an anticonvulsant drug to cure depression and seizures, were analysed. The anaerobically digested and dewatered sewage sludge samples were collected from municipal wastewater treatment plant. Composting experiments were performed under fixed conditions during 30 days. The results of the experiment showed that during a 1-month composting period more than 90% of MET residues degraded, but no degradation of CBZ took place during the composting period. The half-life of MET was 3 days for the compost mixture with the ratios of 1:3 and 1:2 (v:v). The results of this study show that composting maylead to the efficient degradation of MET, whereas for the elimination of CBZ from sewage sludge different means should be used.

Key words: sewage sludge compost, sawdust, fertilizers, metformin, carbamazepine.

INTRODUCTION

The world's pharmaceutical industry has become one of the fastest growing and profitable business sectors. It generates enormous volumes of waste, either directly or indirectly. Hundreds of different active pharmaceutical ingredients (APIs) are discarded in the environment (Agamuthu & Fauziah, 2011). There is clear evidence of impacts of APIs on the building up of bacterial antibiotic resistance (Helwig et al., 2013). As pharmaceuticals are designed to be resistant to biodegradation and current technology at the majority of wastewater treatment plants is unable to remove most APIs, ecosystems are thus exposed to these pollutants (Vallini & Townend, 2010).

Sewage sludge, a residue from the treatment of domestic and industrial wastewater, may be itself regarded as hazardous waste which may cause several undesired consequences due to biological and chemical contaminants, but under certain conditions it can also be used as a fertilizer (Haiba et al., 2016). Taking into account the latter, its safety with respect to pharmaceutical residues (in addition to other potential factors, e.g. pathogens, heavy metals, etc.) must be assessed before use (Kipper et al., 2011). Land application of sewage sludge can be a source of the contamination of food plants by pharmaceutical products (Lillenberg et al., 2010). Plant uptake of pharmaceutical residues, present (even in very small quantities) in soils fertilized with sewage sludge compost, is an obvious reality (Kipper et al., 2011). Although sewage compost is rich in minerals, enabling long-lasting supply for the fast growth of plants (Järvis et al., 2016), antimicrobials consumed even in very small amounts with everyday food can initiate strains of resistant bacteria in human and animal organisms (Kipper et al., 2017). Due to the fact that the use of composted sewage sludge as soil fertilizer is a common practice, knowledge on how the stabilization process affects the reduction of contaminants in this matter is considered crucial (Poluszyńska et al., 2017).

It has been shown that the concentrations of pharmaceuticals decrease after sewage sludge digestion and composting, but they are still present in detectable amounts (Haiba et al., 2016). Amendments of sawdust clearly speed up the degradation of sulfonamides and fluoroquinolones, whereas the mixtures with peat and straw perform lower abilities to decompose the residues of these pharmaceutical (Haiba et al., 2016). In addition to this, sawdust is able to regulate the moisture content and increase the porosity of composting material (Li & Li, 2015). Sawdust has beneficial effects on composting of municipal solid waste. Yousefi et al. (2013) have shown that all compost treatments reached thermophilic temperature rapidly, but the temperature of composting without sawdust showed fluctuations with a rapid drop in the thermophilic temperature and further increase thereafter. On the basis of observed trends in temperature, the composting piles with sawdust required shorter composting periods than those without any sawdust (Yousefi et al., 2013). From an agricultural point of view, sludge co-composted with particularly fine-textured sawdust is the most proper compost material to be applied to soils (Ammari et al., 2012).

The study conducted by Zhong et al. (2018) compared the development of various physicochemical properties and the composition of microbial communities involved in the composting process in the solid fraction of dairy manure (SFDM) with a sawdust-regulated SFDM. The succession of bacteria in both groups proceeded in a similar pattern, suggesting that the effects of the sawdust on bacterial dynamics were minor. Based on this the authors concluded that this confirms the feasibility of composting using only the SFDM. However, this study does not handle the problems associated with different organic pollutants present in dairy manure.

A PhD study was conducted to examine the degradation of some widely used drugs, as fluoroquinolones, sulfonamides, diclofenac (DFC), triclosan (TCS), metformin (MET) and carbamazepine (CBZ) during composting processes, using several bulking agents and different ratios of sewage sludge and sawdust in the mixture (Haiba, 2017). The results reflecting the degradation of sulfonamides and fluoroquinolones, DFC and TCS have been published recently (Haiba et al., 2016 and 2017). Higher proportions of sawdust clearly speeded up the degradation of both DFC and TCS. The current paper is to reflect the results obtained in studying the degradation of MET and CBZ during

sewage sludge co-composting with different portions of sawdust, and to compare the outcomes of this work with the results obtained for DFC and TCS, formerly reported in *Agronomy Research* (Haiba et al., 2017) According to the information available from the scientific publications the biodegradation rate of MET is high, whereas the biodegradation of CBZ does not take place (Mrozik & Stefańska, 2014; Blair et al., 2015; Butkovskyi et al., 2016). DFC readily biodegrades in agricultural soils, whereas the degradation of TCS only partly follows this pathway (Xu et al., 2009).

MET is the first-line medication for the treatment of type 2 diabetes (Maruthur et al., 2016). This disease affects more than 200 million people worldwide (Reitman & Schadt, 2007; Trautwein & Kümmerer, 2011). The results published in 2015 by Niemuth & Klaper demonstrated that MET acts as an endocrine disruptor at environmentally relevant concentrations (Haiba, 2017). Unlike many pharmaceutical drugs, MET is not metabolized by humans but passes unchanged through the body. With no natural degradation processes, MET can be easily reintroduced to humans as they enter the food chain (Trautwein et al., 2014). Detection of MET in seawater and tap water proved the absence of an efficient degradation process in ocean environments or drinking water preparation which suggests a high persistence and the potential for ubiquitous distribution (Trautwein et al., 2014; Haiba, 2017). During sewage treatment a significant reduction of MET concentrations is observed which seems to be mainly due to microbial degradation. Despite the high removal efficiency of sewage treatment plants (STPs), MET is still released in significant amounts into the aquatic environment (Scheurer et al., 2009). MET is a mobile compound with low affinity to soils (Mrozik & Stefańska, 2014). This indicates that this drug may be a potential threat to ground and surface water (Benotti & Brownawell, 2007; Haiba, 2017).

CBZ, an antiepileptic drug, is one of the most frequently detected pharmaceuticals in soil and aquatic environments (Zhang et al., 2008; Oosterhuis et al., 2013). CBZ is used for the treatment of seizure disorders, for relief of neuralgia, and for a wide variety of mental disorders. Approximately 72% of orally administered CBZ is absorbed, while 28% is unchanged and subsequently discharged through the feces (RxList4; Zhang et al., 2008; Haiba, 2017). Nieto et al. (2010) determined concentrations between 11 and 42 mg kg^{-1} (dry weight – dw) for CBZ in samples from two STPs. However Miao et al. (2005) detected CBZ at concentration 69.6 µg kg^{-1} (dw) in untreated biosolids and at concentration 258.1 µg kg^{-1} (dw) in treated biosolids. Chefetz et al., 2008 indicated that CBZ exhibits the persistence characteristic of organic contaminants, potentially leading to long-term environmental risks (Haiba, 2017). It is known that CBZ is toxic for some algae, bacteria, invertebrates and fish (Camacho-Munoz et al., 2010). There are no conclusive results confirming the effects (or their lack) of prolonged exposure of organisms to low concentrations of CBZ (Rezka et al., 2015; Haiba, 2017).

CBZ is highly persistent and frequently found in sewage, surface waters and managed aquifer recharge systems (Leclercq et al., 2009; Nieto et al., 2010), and once it is discharged into the environment it causes toxicity (Joss et al., 2006; Verlicchi et al., 2012). Removal of CBZ and its metabolites from municipal sewage treatment plant is very low (~8%). CBZ is persistent in soils (Li et al., 2013; Grossberger et al., 2014; Paltiel et al., 2016) and has been shown to be taken up and accumulate in a variety of crops (Winker et al., 2010; Shenker et al., 2011; Holling et al., 2012; Goldstein et al., 2014; Malchi et al., 2014, Haiba, 2017). CBZ is recalcitrant both in biodegradation and photolysis experiments. This compound is retained by the soil where it is accumulated

due to its low degradation rate. Slow degradation rate coupled with plant uptake phenomenon indicates that CBZ present in biosolids amended soils is a significant concern and potential risk (Durán-Álvarez et al., 2015). Researchers have quantified acute toxicity of CBZ < 100 mg L^{-1} (Malarvizhi et al., 2012; Haiba, 2017).

MATERIALS AND METHODS

The procedures described in the current section are identical to those presented in Haiba et al., 2017 with the exception that the drugs used were different: instead of examining the concentration changes of DFC and TCS, the degradation of CBZ and MET was studied during sewage sludge composting. The composting parameters were in excellent agreement with those presented in Haiba et al., 2017, showing the efficiency of the composting process.

Chemicals and materials

CBZ (99.9%) and MET hydroxide (99.8%) were obtained from Sigma-Aldrich. LC-MS eluent components were: 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). The samples were prepared using 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 preparation

The anaerobically digested and subsequently dewatered by centrifugation sewage sludge samples were obtained from a municipal wastewater treatment plant. Prior to the treatment by aerobic composting under laboratory conditions the sewage sludge was mixed with sawdust at two different ratios (1:2 and 1:3 sludge: sawdust, v:v). The initial concentration of both CBZ and MET was 2 mg kg^{-1} in relation to dry weight (dw). Reference piles without additions of pharmaceuticals and with the same ratios of sludge and sawdust were prepared. 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. 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 manually to ensure that the solid content is in contact with extraction solvent.
5. Extraction was continued by sonicating during 10 min.
6. Samples were centrifuged at 7,830 rpm during 5 min.
7. The extracts were removed from the tube using pipette.

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 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 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 ethanol. 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. Concentrations of the solutions used for calibration were chosen according to the linear range for each analyte.

LC-MS/MS analysis

Sample extracts were analyzed (as described in Haiba et al., 2017) 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 1.

Table 1. MRM transitions, collision energies (CE) and ionization polarities used for analysis

Analyte	Precursor ion, *m/z*	Product ion, *m/z*	CE	Polarity mode
Carbamazepine	237	194	20	positive
	237	179*	40	positive
Metformin	130	71	25	positive
	130	60	10	positive

* – 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.

RESULTS AND DISCUSSION

Before spiking the (initial) concentrations of MET in the mixtures of sewage sludge and sawdust were very low: 1 to 2 μg kg^{-1} (dw) (Table 2). As it can be seen from Table 2, none of the compost mixtures was free of CBZ. Its concentrations were from 41 to 62 μg kg^{-1} (dw). This data for CBZ is in reasonable agreement with the results published by Miao et al. in 2005 (Haiba, 2017). Similar concentrations with CBZ were found in compost mixture before spiking for DFC (64 to 86 μg kg^{-1} dw) (Haiba et al., 2017).

Table 2. Concentrations of metformin and carbamazepine in sewage sludge–sawdust mixtures (mg kg^{-1}, dw)

Compound	Mixture ratio (v:v)	Before spiking	1 day	1 week	1 month
Metformin	1:2	0.002 ± 0.000	2.14 ± 0.25	0.44 ± 0.02	0.18 ± 0.01
	1:3	0.001 ± 0.000	1.95 ± 0.15	0.30 ± 0.02	0.14 ± 0.02
Carbamazepine	1:2	0.062 ± 0.002	3.11 ± 0.38	2.59 ± 0.05	3.20 ± 0.10
	1:3	0.046 ± 0.003	2.69 ± 0.26	2.31 ± 0.08	2.32 ± 0.08

lower detection limit for MET – 0.009 ng mL^{-1}; for CBZ – 0.004 ng mL^{-1} in injected solution.

After preparing compost mixtures unexpectedly high concentrations of CBZ were detected. This phenomenon can be explained with the rapid loss of organic matter during the initial stage of composting and is in agreement with the results obtained by Blair et al. (2015), which showed that the concentrations of CBZ and its metabolites increased on a dry weight basis between untreated and treated biosolids. It has been also established that in wastewater treatment plants CBZ sometimes exhibits negative removal efficiency (Collado et al., 2014, Haiba, 2017).

The results measured after 1 week showed that MET had decreased by 79% 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 drop was only 85%.

The results given in Tables 2 and 3 showed that no degradation of CBZ took place, whereas over 90% of MET degraded during a 1-month composting (Haiba, 2017). Butkovskyi et al. (2016) have shown that under specific conditions the partial degradation of CBZ takes place. CBZ is not mineralized in soil but is transformed to a range of transformation products, especially to the recalcitrant acridone-*N*-carbaldehyde (Li et al., 2013). The degradation products of CBZ are more toxic than CBZ (Donner et al., 2013). The formation of these products might also take place during sewage sludge composting (Butkovskyi et al., 2016; Haiba, 2017).

Table 3. Extent of degradation (%) for metformin and carbamazepine during one week and month composting

Sample No	Mixture ratio (v:v)	Metformin		Carbamazepine	
		1 week	1 month	1 week	1 month
1	1:2	79	91	16	-11
2	1:3	85	93	14	13

CBZ readily adsorbs on sludge particles (Blair et al., 2015; Nielsen & Bandosz, 2016; Haiba, 2017). The work carried out by Koba et al. (2016) showed that CBZ and its metabolites are persistent under the studied conditions in soils. According to Li et al. (2013) the values of $t_{1/2}$ for CBZ in soils were between 46 and 173 days (in the studied mixtures $t_{1/2}$ was 178 to 222 days.

CBZ was an exception in the study: this compound was persistent under all studied conditions. According to Collado et al. 2014 in some cases CBZ exhibits even negative removal efficiency with no seasonal variation (Golovko et al., 2014). The results showed this same phenomen in this study (see Table 4). This leads to the conclusion that composting is not an appropriate mean for degrading this compound.

Table 4. The degradation rate constant and half-lives of carbamazepine and metformin

Compound	Mixture ratio (v:v)	Current study			Data from literature		
		k(d^{-1})	$t_{1/2}$ (d)	%	k(d^{-1})	$t_{1/2}$ (d)	%
CBZ	1:2	0.00	222	-11		46…173[a]	
	1:3	0.00	178	13			
MET	1:2	0.22	3	91	0.12–0.26[a]	1–5[a]	99–100[a]
	1:3	0.27	3	93	0.22–0.27[b]	2–3[b]	

[a] – agricultural soil, [b] – compost mixture; CBZ – Li et al. (2013); MET– Mrozik & Stefańska, 2014.

For comparison, 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 (Haiba et al., 2017). TCS gives the following k and $t_{1/2}$ values in the case of agricultural soils (Xu et al., 2009; Haiba, 2017): k = 0.05–0.04 d^{-1}; $t_{1/2}$ = 13–20 d. In sterile soil k = 0.02 d^{-1} and $t_{1/2}$ = 35 d; 45% of TCS degrades during 30 days. In the case of compost mixtures k = 0.03–0.05 d^{-1} and $t_{1/2}$ = 13–26 d. The level of degradation was 55–81%. TCS readily adsorbs on soil particles and due to this its mobility in soils is low (Xu et al., 2009; Haiba, 2017). Bioavailability of TCS greatly decreases in biosolids-amended soils. Biosolids decrease plant uptake primarily by increasing soil organic carbon content and subsequently sorption (Fu et al., 2016; Haiba, 2017).

Results of this study and results presented in Haiba et al. (2017) showed clearly that the degradation of both MET (93%) and DCF (98%) almost fully takes place already during one-month composting period in the case of compost samples with the ratios of 1:3 (v:v). According to Mrozik & Stefańska (2014) MET appears to be a highly mobile compound with a low affinity to soils (K_d = 1.4–0.5 mL g_{ss}^{-1} for MET in different soils). MET is polar and very soluble in water; thus it interacts more strongly with water than with the soil surface. Although the half-lives of MET were 1–5 days in different soils (Table 4), due to its weak sorption MET may be a potential threat to ground and surface water (Benotti & Brownawell, 2007; Haiba, 2017). The degradation of MET takes place

rapidly and fully both in soils (from Mrozik & Stefańska, 2014: $k = 0.12–0.26$ d^{-1}; $t_{1/2} = 1–5$ d) and compost mixtures ($k = 0.22–0.27$ d^{-1}; $t_{1/2} = 2–3$ d). According to Markiewicz et al. (2017) in most cases MET follows a dead-end pathway with formation of guanylurea. The formed guanylurea does not degrade any further and also does not show toxic properties. In the case of different soils there is a 99–100% degradation of MET during a 30-day period (Mrozik & Stefańska, 2014), whereas in the studied compost mixture degradation was lower at 92–93% (Table 4).

Similarly, DCF is not persistent and is readily biodegradable in soil; its degradation follows the first-order exponential decay model and half-life ($t_{1/2}$) is ranging from 0.4 to less than 5 days (Xu et al., 2009; Al-Rajab et al., 2010; Dalkmann et al., 2012; Carter et al., 2014; Grossberger et al., 2014; Haiba, 2017). The bioconcentration factors found for DCF were high in the case of long-term irrigation with sewage (Christou et al., 2017). In agricultural soils (Xu et al., 2009) $k = 0.23–0.16$ d^{-1}and $t_{1/2} = 3–4$ d. In the case of sterile soil $k = 0.01$ d^{-1} and $t_{1/2} = 70$ d (Xu et al., 2009), and for compost mixtures $k = 0.09–0.1$ d^{-1} and $t_{1/2} = 7–8$ d (Haiba, 2017). According to this data in sterile soil only 26% of DCF degrades during a 30-day period, whereas in compost mixtures the level of degradation was 92–98%. This leads to the conclusion that the biodegradation of DCF prevails over its chemical degradation.

Data obtained as a result of degradation experiments were fitted to the exponential decay model: $C = C_0e^{-kt}$ to obtain the degradation rate constant k. Half-lives ($t_{1/2}$) were calculated by the equation: $t_{1/2} = 0.693/k$ (Xu et al., 2009; Haiba, 2017).

As a rule, the degradation rate of pharmaceuticals depends on the media consistency. In agricultural soils biodegradation of pharmaceuticals is faster than in freshly made compost mixtures probably due to the fact that the formation of microbial communities in the latter presumably takes time. Strong adsorption of pharmaceuticals to soil or sludge particles inhibits the degradation of pharmaceuticals. At the same time, this also slaps down the plant uptake of these pharmaceuticals, which is important in the view of food safety (Haiba, 2017).

CONCLUSIONS

This study was carried out to demonstrate the degradation of CBZ and MET in composting processes using different ratios of sewage sludge and bulking agent (sawdust). In the case of MET, compost samples with the sludge-sawdust ratios of 1:3 and 1:2 (v:v) yielded similar degradation of more than 90% during a 1-month composting period. No degradation of CBZ takes place during composting experiments.

The current study (involving MET and CBZ) and the results (for DFC and TCS) published in Haiba et al. (2017) leads to the conclusion that composting might ensure the efficient degradation of DCF, MET and TCS, whereas for the elimination of CBZ from sewage sludge different means should be used. The persistence of pharmaceuticals increases in the following line: MET→DFC→TCS→CBZ.

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