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Fluoroquinolones and sulfonamides in sewage sludge compost and their uptake from soil into food plants

Egge Haiba[1], Merike Lillenberg[2], Karin Kipper[3], Alar Astover[4], Koit Herodes[3], Mari Ivask[1], Annely Kuu[1], Sandra Victoria Litvin[1] and Lembit Nei[1]*

[1]Tartu College, Tallinn University of Technology, Puiestee 78, 51008 Tartu, Estonia.
[2]Estonian University of Life Sciences, Kreutzwaldi 58A, 51014 Tartu, Estonia.
[3]Institute of Chemistry, University of Tartu, Ravila 14A, 51010 Tartu, Estonia.

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Sewage sludge compost can be a source of nutrients for plants and contamination by pharmaceutical products. In this study the presence of some widely used pharmaceuticals in sewage sludge and its compost – namely ciprofloxacin C17H18FN3O3, ofloxacin C18H20FN3O4, norfloxacin C16H18FN3O3, sulfadimethoxine C12H14N4O4S and sulfamethoxazole C10H11N3O3S – was shown. In several sewage sludge samples their concentrations exceeded the relevant trigger values for manure. The highest concentrations of ciprofloxacin, ofloxacin and norfloxacin in the compost ready for commercialization sufficiently exceeded the threshold concentration – 1 µg/kg – for pharmaceuticals in soil. The values of the highest detected concentrations of these pharmaceuticals in compost were respectively 70, 64 and 8 µg/kg. The uptake of these pharmaceuticals was demonstrated from both sandy and loamy soils into food plants such as carrot (*Daucus carota* L), potato (*Solanum tuberosum* L) and wheat (*Triticum vulgare* L).

Key words: Soil pollution, plant uptake, pharmaceuticals.

INTRODUCTION

Utilization of sewage sludge for agricultural application is increasing (Babel et al., 2009; Lillenberg et al., 2009). Composting is recognized as one of the sewage sludge recycling options (Hara and Mino, 2008). The scientific community has become increasingly interested in the impacts of pharmaceutical contaminants to the environment and human health, leading to the development of novel analytical tools (Kipper et al., 2011). In contrast to the properties and effects desired from the therapeutic application of antibiotics, these same properties are often disadvantageous for those target and non-target organisms present in the environment. The primary route of entry of human pharmaceuticals into the environment is through sewage point sources. Pharmaceuticals may be transferred without degradation and stored, at least temporarily, in other matrices or compartments through processes such as bio-concentration, sorption and deposition of particles (Glassmeyer et al., 2008).

Composting of organic wastes is a traditional way to reuse organic matter (Tremier et al., 2005; Suthar and Sing, 2008). Previous studies have shown (Büyüksönmez and Sekeroglu, 2005), that the degradation of some pharmaceuticals (ibuprofen, galaxolide) and personal care products (phthalate esters) may take place during

*Corresponding author. E-mail: lembit.nei@ttu.ee.

bio-solid composting, but still no systematic work concerning the degradation of antimicrobials during sewage sludge composting has been published. It has been claimed, that the content of antimicrobials in the compost made from sewage sludge may easily lead to their elevated concentrations in food plants, if the compost is used as a fertilizer (Lillenberg et al., 2010). A number of pharmaceuticals, known to be persistant in soil, are able to accumulate into food plants (Brambilla et al., 1996; Jjemba, 2002; Migliore et al., 2003; Boxall et al., 2006; Dolliver et al., 2007).

Remarkable amounts of pharmaceuticals enter the soil via fertilizing with sewage sludge. There exist no trigger values for residues of human pharmaceuticals in sewage sludge or its compost in European Union. The most closely related act is EU directive establishing trigger values for veterinary medicines in manure (EMEA/CVMP/055/96, 1998). The content of drugs should not exceed 100 µg/kg in manure, and 10 µg/kg in soil fertilized with manure. However, the EU Scientific Steering Committee (EU SSC) considers the trigger value for pharmaceuticals in soil non-scientific and recommends a value considerably lower - 1 µg/kg. Only such concentration can be safe for all soil organisms (Montforts, 2005).

The antibiotic resistance in soil bacteria can develop even at lower drug concentration in soil. This would push the soil concentration trigger further down to 0.01 to 0.1 µg/kg (Montforts, 2005). The antibiotic resistance can be transferred from soil bacteria to pathogens via horizontal gene transfer (Knapp et al., 2010). The trigger values recommended by EMEA/CVMP and EU SSC were used for estmation of the safety of sewage sludge and its compost as agricultural fertilizer.

The aim of this work was to study the presence and concentration levels of some widely used fluoroquinolones and sulfonamides in urban sewage sludge and its compost, and the possible uptake of these antimicrobials from compost-fertilized soils into food plants.

MATERIALS AND METHODS

Chemicals and equipment

In the current study the selection of pharmaceuticals was made considering their possible presence in sewage sludge compost, stability in soil and potential ability to accumulate into plants. These pharmaceuticals included fluoroquinolones (FQs), ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFL), and sulfonamides (SAs), sulfadimethoxine (SDM) and sulfamethoxazole (SMX). FQs and SAs represent the most commonly used families of antibiotics (Pérez et al., 2005; Picó and Andreu, 2007). FQs are among the most important antibacterial agents used in human and veterinary medicine. Because of the growing practice of adding manure and sewage to agricultural fields these drugs end up in soils, where they can accumulate and have adverse effects on organisms. CIP is the most widely prescribed FQ in the world, followed by OFL. NOR, an oral broad-spectrum antibacterial agent is very common in Europe (Picó and Andreu, 2007). SAs are among the most commonly used antibiotics in veterinary medicine and to a lesser extent in human medicine (García-Galán et al., 2009). In the present study, SDM and SMX were chosen as target antibiotics because of their widespread use (Isidori et al., 2005; De Liguoro et al., 2007). SMX is one of the most consumed SAs in human medicine. It has been reported frequently and is considered ecologically harmful (García-Galán et al., 2009).

Antibiotics were purchased from Riedel-de-Haën (Seelze, Germany) - three FQs: CIP (purity 99.8%), NOR (purity 99.9%) and OFL (purity 99.3%); two SAs: SDM (purity 99.4%) and SMX (purity 99.9%). Hydrophilic-lipophilic balanced (HLB) cartridges (Oasis HLB (60 m), 500 mg / 6 ml) by Waters (Milford, MA, USA). Acetonitrile and methanol were obtained from J.T. Baker (Deventer, The Netherlands), phosphoric acid from Lachema (Brno, Czech Republic), citric acid monohydrate from Fisher Scientific (Pittsburgh, PA, USA), formic acid from Riedel-de-Haën, ammonium acetate from Fluka (Buchs, Germany). All solvents were of reagent grade or higher quality.

Collection of the sewage sludge and compost samples

"Raw" sewage sludge, 6 and 12 months stored compost were sampled. Approximately 200 g of sludge (content of dry matter was 28% in Tallinn and 25% in Tartu) or sewage sludge compost (anaerobically digested sludge mixed with peat in Tallinn or pressed raw sludge mixed with tree bark in Tartu) was placed into a 500 ml glass jar and mixed thoroughly. The jar was covered hermetically with a lid. The samples were stored at +4°C in the dark to avoid photodegradation of antimicrobials. The samples were analyzed as soon as possible, typically within a week. Alternatively they were stored in polypropylene vials frozen at temperature -80°C.

Determination of antimicrobials from sewage sludge and compost

The methodology used for the determination of antimicrobials from sewage sludge and compost together with method validation is described in detail by Lillenberg et al. (2009). Pressurized liquid extraction (PLE) followed by solid phase extraction (SPE) and liquid chromatography electrospray ionization – mass spectrometry (LC-ESI-MS) were used for analysis. Relative standard deviation (RSD) of the determinations was within 2%.

Plant uptake experiments

Potato (*Solanum tuberosum* L.), carrot (*Daucus carota* L.) and wheat (*Triticum vulgare* L.) were grown in the presence of five antimicrobials, found in Estonian sewage sludge (CIP, NOR, OFL, SDM, and SMX). The potato tubers or seeds of plants were planted into the pots with the capacity of 3 dm3, one tuber or 35 seeds in every pot. The plants were cultivated in greenhouse under natural light conditions for 120 days from planting. Two different soils were used for experiments - loamy and loamy sand. The soil was weighted and aqueous solutions of the studied pharmaceuticals were mixed with soil.

The final concentration of each pharmaceutical in soil was 0.01; 0.1; 0.5; 1 and 10 mg/kg (dry weight). To assure better dissolution of the studied pharmaceuticals FQs were dissolved in 2 ml of 0.1 mM ammonium acetate buffer solution with pH=2.8 and SAs were dissolved in 2 ml of 0.3 M NaOH. Three parallel pots were used for each concentration of antimicrobials in both soils, and for control plants grown in antimicrobial-free soil. The edible parts of the plants were collected, washed carefully, dropped, dried in the dark and milled for analyses. The milled samples were dried in a thermostate

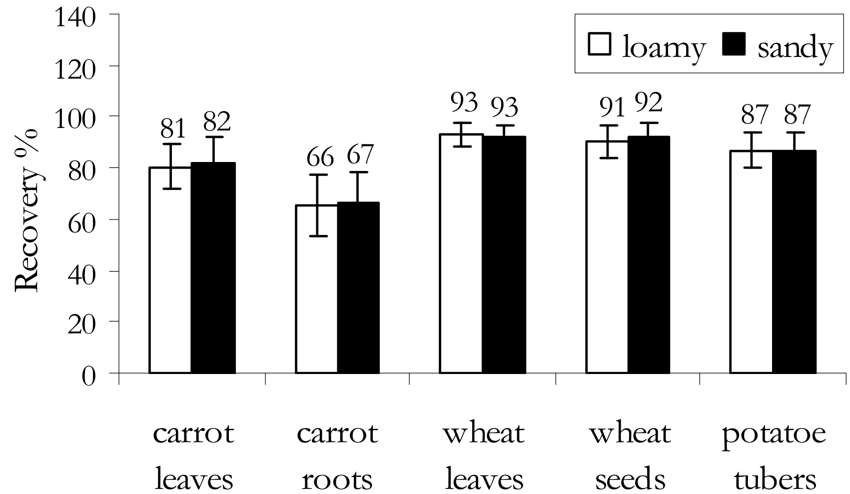

Figure 1. Average recoveries for 5 antimicrobials (CIP, NOR, OFL, SDM, SMX) detected from different parts of food plants grown in different soils using LE and SPE. Error bars show the recovery ranges

at 45°C and held in hermetic plastic bags at -80°C before analysis.

Determination of antimicrobials from plants

Liquid extraction

Determination of antibiotic residues in plant material has been demonstrated in Kipper et al. (2011). Method for liquid extraction was modified from Palmada et al. (2000). 250 mg of dried plant sample was extracted with 10 ml of 1:1 (v/v) mixture of acetonitrile and 1% acetic acid, then homogenized with laboratory homogenizer DIAX 900 (Heidolph Instruments, Germany) 25 000 rpm, sonicated (5'), vortexed (1') and centrifuged at 8000 rpm. The supernatant was separated and dried by nitrogen stream. 15 ml of 1% acetic acid was added to the 1 ml of evaporation residue.

Solid phase extraction

The extract collected by liquid extraction was cleaned up by solid phase extraction (SPE). Antibiotics - CIP, NOR, OFL, SDM and SMX - were extracted using HLB cartridges. For SPE procedure the vacuum manifold, supplied by Agilent Technologies, was used. HLB cartridges were preconditioned with 20 ml of methanol and 10 ml of Milli-Q water. The sample was loaded at a rate of 6 ml/min. After extraction, the compounds were eluted from cartridges using 12 ml of methanol. The SPE extracts were concentrated in polypropylene vials in N_2 stream. Residue was dissolved in 1 ml of 10% methanol with a buffer solution (5 mM 1,1,1,3,3,3-hexafluoro-2-propanol, pH adjusted to 9.0 with NH_4OH).

LC-ESI-MS method for detection of antimicrobials from plants

The SPE extracts were analyzed by liquid chromatography electrospray ionization - mass spectrometry (LC-ESI-MS). Antimicrobials were chromatographed using a Waters X Bridge C18 column (150 × 3 mm, 3.5 µm) equipped with a Waters Guard Cartridge 4.6 × 20 mm. Gradient elution was carried out with methanol and hexafluoroisopropanol (HFIP) buffer solution (5 mM 1,1,1,3,3,3-hexafluoro-2-propanol, pH adjusted to 9.0 with NH_4OH). The linear gradient started at 10% methanol and was raised to 100% within 50 min, after that methanol concentration was 100% for 5 min, then lowered to 10% in 5 min and kept in 10% for 5 min. The eluent flow rate was 0.3 ml/min, the column temperature was set to 30°C and the injection volume was 10 µl.

Method validation

The described method was validated for the simultaneous determination of CIP, NOR, OFL, SDM, and SMX from plants. For calibration antimicrobials standard solutions were prepared in eluent (hexafluoroisopropanol and 10% methanol). The calibration graphs with peak area versus concentration were composed on concentration range 1 to 10 000 ng/ml and were linear with $r2>0.9998$. Recovery was calculated from standard addition experiments. Recoveries for all detected pharmaceuticals in all matrices varied from 54 to 98%, the average recoveries are shown in Figure 1. The method validation was performed in the matrix, which showed the lowest recovery – carrot roots in loamy soil (recovery ranges 54 to 78%, average recovery 66%) (Figure 1). The average recoveries of antimicrobials from carrot roots were 73% (CIP), 69% (NOR), 76% (OFL), 55% (SDM), 70% (SMX). Standard deviations for the recoveries were 1% (CIP), 2% (NOR), 2% (OFL), 1% SDM and 1% SMX.

The limits of quantification (LOQ) were as follows: CIP 108.3; NOR 162.2; OFL 22.9; SDM 71.2 and SMX 130.6 µg/kg. The standard deviations were accordingly 2.7; 4.1; 0.6; 1.8 and 3.3. LOQ was estimated as 10 times of the standard deviation from five replicate analysis of unspiked and spiked plant samples using HLB cartridges.

RESULTS AND DISCUSSION

Pharmaceuticals in sewage sludge compost

As shown in Tables 1 and 2, the concentrations of the studied antimicrobials decreased during composting. In Tallinn the antimicrobials were almost absent in compost

Table 1. The highest contents of antimicrobials in Tallinn sewage sludge and its compost.

AM	Concentration µg/kg (dm)			
	Sewage sludge	6 months stored compost	12 months stored compost	Trigger value for soil
CIP	1520	9	0.3	10*
NOR	580	17	0.1	
OFL	134	8	0.03	
SDM	73	n.d.	n.d.	1**
SMX	22	n.d.	0.01	

AM, antimicrobial; CIP, ciprofloxacin; NOR, norfloxacin; OFL, ofloxacin; SDM, sulfadimethoxine; SMX, sulfamethoxazole; *recommended by EMEA/CVMP; ** recommended by EU SSC; n.d., not detected.

Table 2. The highest contents of antimicrobials in Tartu sewage sludge and its compost.

AM	Concentration µg/kg (dm)			
	Sewage sludge	6 months stored compost	12 months stored compost	Trigger value for soil
CIP	442	44	70	10*
NOR	439	40	64	
OFL	157	9	8	
SDM	32	1	n.d.	1**
SMX	16	2	2	

AM, antimicrobial; CIP, ciprofloxacin; NOR-norfloxacin; OFL, ofloxacin; SDM,sulfadimethoxine; SMX-sulfamethoxazole; *recommended by EMEA/CVMP; ** recommended by EU SSC; n.d. - not detected.

stacks that had been formed 12 months earlier. However, in the compost stored for 6 months the contents of all FQs exceeded the trigger value for soil recommended by EU SSC and the content of NOR exceeded both triggers. In Tallinn the 6-months stored compost is ready for application, in Tartu the storage time must be at least one year. In Tartu the antibiotics were not completely degraded even after 12 months of storage of the compost stack (Table 2). The contents of CIP and NOR were remarkably higher than the trigger values for soil. The contents of OFL and SMX were lower, but still exceeded 1 µg/kg. SDM was not detected in the compost stored for 12 months. Despite mixing, the compost was not homogeneous. The concentrations of pharmaceuticals vary noticeably within the same compost stack. For example, the content of fluoroquinolones differed up to 1.8 times within the same stack in Tartu. Heterogeneity of the compost may be the result of adsorption of the pharmaceuticals to solid particles (Carmosini and Lee, 2008).

We suppose that the main reason of the decrease in pharmaceutical concentrations during composting is the applied sludge treatment technology. The decomposition of pharmaceuticals was faster in the case of Tallinn composting technology. In Tartu the sewage sludge compost was made by mixing the raw sludge with tree bark, in Tallinn the methane fermentation and mixing with peat were used. The compost stacks were mixed frequently in both cities for promoting growth of the aerobic bacteria. Mixing exposes different parts of the stack to the light. As photodegradation is considered to be one of the reason of decomposition of FQs (Hooper and Wolfson, 1991), the time of stack mixing might have an influence to the degradation rate of FQs.

Uptake of pharmaceuticals by food plants

At soil concentrations of 10 mg/kg antimicrobials accumulated in potato tubers and carrot roots in amounts, which exceeded their maximum residue levels (MRL) set for food of animal origin - milk and meat (EMEA/MRL/026/95; EMEA/MRL/820/02, 2002). The highest concentrations of antimicrobials accumulated in plants are shown in Table 3.

Plants accumulated antimicrobials from soil even at soil concentration of 0.01 mg/kg (CIP, OFL). The drug residues were detected in carrot roots and potato tubers. CIP, OFL and SDM were detected also in wheat seeds. The level of accumulation depended on chemical properties of the compound, soil type, plant species and part (overground or underground). As a rule, the higher concentrations of antimicrobials were detected in the plants grown in sandy soil. The average contents of antimicrobials in edible parts of the plants grown at lower drug concentrations (1 mg/kg) were higher than MRL in case of OFL, SDM and SMX in carrot roots. The MRL for SAs –100 µg/kg - is set for the sum of all SAs in meat

Table 3. The highest contents of antimicrobials detected in edible parts of food plants µg/kg (dm).

AM	AM conc. in soil mg/kg (dm)	Carrot roots		Potato tubers		Wheat seeds		MRL for milk and meat (µg/kg)
		Loamy	Sandy	Loamy	Sandy	Loamy	Sandy	
CIP	10	-	740	170	160	-	†	100
	1	-	50	20	10	-	-	
	0.5	-	70	-	50	40	-	
	0.1	-	-	40	6	-	-	
	0.01	-	-	-	3	-	-	
NOR	10	-	990	180	260	-	†	-
	1	-	80	40	-	-	-	
	0.5	-	-	-	-	-	-	
	0.1	-	-	40	-	-	-	
OFL	10	40	830	110	240	-	†	-
	1	-	160	60	50	-	9	
	0.5	30	80	30	90	30	-	
	0.1	5	10	6	20	15	5	
	0.01	3	10	3	5	-	-	
SDM	10	100	660	340	1750	50	†	SDM + SMX
	1	130	20	120	40	-	-	
	0.5	40	10	-	10	36	-	
SMX	10	480	4910	580	5150	-	†	100
	1	120	290	-	-	-	-	
	0.5	60	110	-	-	-	-	
	0.1	-	20	-	-	-	-	

AM-antimicrobial; CIP-ciprofloxacin; NOR-norfloxacin; OFL-ofloxacin; SDM-sulfadimethoxine; SMX-sulfamethoxazole; MRL- maximum residue level; †- at soil AM concentration 10 mg/kg the wheat plants wilted before flowering.

and milk (EMEA/MRL/026/95, 1995). In carrot roots the sum of average concentrations of SDM and SMX was over the MRL. CIP, OFL and SDM were detected in wheat seeds grown in loamy soil, however, in wheat seeds grown in sandy soil only OFL was found. The level of germination of the wheat seeds in sandy soil at antimicrobial concentration of 10 mg/kg was very low and the development of the plants was noticeably slowed down. These plants wilted before flowering and the formation of grains could not take place. In carrot roots and potato tubers most of the studied antimicrobials were detected, except CIP and NOR in carrots grown in loamy soil. OFL accumulated into carrots and potatoes from soils with lowest antimicrobial concentration - 0.01 mg/kg. The content of CIP was found only in potatoes grown in sandy soil at antimicrobial concentration of 0.01 mg/kg.

The content of antimicrobials in plants cultivated in sandy soil was usually higher than in plants grown in loamy soil. Potato tubers and carrot roots grown in sandy soil at highest drug concentration of 10 mg/kg contained several hundreds or thousands micrograms of antimicrobials per kg. The content of antimicrobials in potatoes and carrots grown in loamy soil was considerably lower. SAs are among the most commonly used antibiotics in veterinary medicine and to a lesser extent in human medicine (Thiele-Bruhn, 2003). They are both fairly water-soluble and polar (Thiele-Bruhn et al., 2004).

The low adsorption of SAs on soil particles is known (Beausse, 2004) and due to this phenomenon they are “ready” to migrate into plants. An opposite behavior is characteristic to FQs. It has been shown that more than 90% of applied CIP and OFL is adsorbed on different soils (Beausse, 2004). For this reason no significant migration of FQs from soil into plants takes place. In loamy soil the molecules of SAs attach to clay particles (Thiele-Bruhn, 2003), reducing their uptake by plants.

Variance analysis (ANOVA) showed that plant uptake results were statistically significant ($p<0.05$) only in the case of carrot roots and potato tubers grown in soils with drug concentrations of 10 mg/kg. At lower drug concentrations in soil the dispersion of the results was too high, which can be explained with the very high heterogeneity of both soil and plant matter.

Conclusions

FQs and SAs were present in sewage sludge and its compost both in Tallinn and in Tartu and in several samples their concentrations exceeded the relevant trigger values for manure. Degradation of these pharmaceuticals took place as a result of composting. The concentrations of the studied antimicrobials decreased remarkably as a result of composting. Still, in 6 month stored compost the content of NOR was over and the content of CIP was near the recommended trigger value for soil. The decomposition rate of pharmaceuticals depends on the applied sludge treatment technology. The decomposition of pharmaceuticals was faster in the case of Tallinn composting technology.

The uptake of pharmaceuticals by the studied food plants was present. Wheat grains had low or zero concentrations of the analysed pharmaceuticals. This shows the potential applicability of sewage sludge compost for fertization of the crops of this type. The uptake of FQs and especially SAs by plants like potato and carrot might present health risk. Due to this the application of sewage sludge as a fertilizer for these crops may take place only after careful testing against possible different toxic pollutants. The safest way to exclude exposing plants to pharmaceuticals is to ensure that these substances are adequately degraded before sewage sludge compost is applied onto arable land.

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