THESIS ON CIVIL ENGINEERING F71

Optimization of Sewage Sludge Composting: Problems and Solutions

EGGE HAIBA

TTÜ PRESS

TALLINN UNIVERSITY OF TECHNOLOGY
School of Engineering
Tartu College

This dissertation was accepted for the defence of the degree of Doctor of Philosophy in Civil and Environmental Engineering on 10 October 2017.

Supervisor: Prof. Lembit Nei
Tallinn University of Technology, Tartu College, ESTONIA

Co-supervisor: Dr. Merike Lillenberg
Estonian University of Life Sciences, ESTONIA

Opponents: Dr. Henry A. Alegria
University of South Florida St. Petersburg, USA

Dr. Olga Hogan
LiveHub Pty Ltd, AUSTRALIA

Defence of the thesis: 8th December 2017, Tallinn

Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for any academic degree.

/Egge Haiba /

ISSN 1406-4766
ISBN 978-9949-83-179-1 (publication)
ISBN 978-9949-83-180-7 (PDF)

EHITUS F71

Reoveesette kompostimistehnoloogiate optimeerimine ravimijääkide kahjutustamise eesmärgil

EGGE HAIBA

TTÜ
KIRJASTUS

To my mother Eiri

CONTENTS

LIST OF ORIGINAL PUBLICATIONS THAT CONSTITUTE THE THESIS

The thesis is based on the following academic publications, which are referred to in the text by numbers I to VII:

I **Haiba, E.**, Nei, L., Kutti, S., Lillenberg, M., Herodes, K., Ivask, M., Kipper, K., Aro, R., Laaniste, A. (2017). Degradation of diclofenac and triclosan residues in sewage sludge compost. *Agronomy Research*, 15 (2), 395−405.

II Kipper, K., Lillenberg, M., Herodes, K., Nei, L., **Haiba, E**. (2017). Simultaneous determination of fluoroquinolones and sulfonamides originating from sewage sludge compost. *The Scientific World Journal*, 2017, 8p.

III **Haiba, E**., Nei, L. (2017). Sewage sludge composting and pharmaceuticals. *European Scientific Journal*, 114−121.

IV **Haiba, E**., Nei, L., Ivask, M., Peda, J., Järvis, J., Lillenberg, M., Kipper, K., Herodes, K. (2016). Sewage sludge composting and fate of pharmaceutical residues – recent studies in Estonia. *Agronomy Research*, 14 (5), 1583−1600.

V Nei, L., **Haiba, E**., Kutti, S., Lillenberg, M., Kipper, K., Herodes, K. (2014). Sewage sludge compost, microbial activity and pharmaceuticals. New Trends and Issues Proceedings on Advances in Pure and Applied Sciences, *Global Journal on Advances Pure and Applied Sciences,* North America, 312 09 2014, 30−37.

VI **Haiba, E**., Nei, L., Lillenberg, M., Kipper, K., Herodes, K. (2013). Degradation of some pharmaceuticals during sewage sludge composting. *Global Journal on Advances Pure and Applied Sciences*, North America, 123 07 2013, 1, 857−862.

VII **Haiba, E**., Lillenberg, M., Kipper, K., Astover, A., Herodes, K., Ivask, M., Kuu, A., Litvin, S.V., Nei, L. (2013). Fluoroquinolones and sulfonamides in sewage sludge compost and their uptake from soil into food plants. *African Journal of Agricultural Research*, 8 (23), 3000−3006.

The original articles have been reprinted with kind permission from the *Agronomy Research* (I, IV), *The Scientific World Journal* (II), *European Scientific Journal* (III) and *African Journal of Agricultural Research* (VII).

AUTHOR'S CONTRIBUTION TO THE PUBLICATIONS

Paper	Original idea	Study design and methods	Data collection and handling	Contribution to result interpretation and manuscript preparation	Responsible for result interpretation and manuscript preparation
I	**EH**	**EH**, KH	**EH**, LN, MI	**EH**, RA, AL	**EH**, KH, LN
II	KK	KK	KK, **EH**	**EH**, KK, LN	**EH,** KK, LN
III	LN, **EH**	**EH**, LN	**EH**, LN	**EH**	**EH**, LN
IV	**EH**, LN	**EH,** LN	**EH,** LN, MI	**EH**, JP, JJ	**EH,** LN
V	**EH**	**EH**, SK	**EH**, SK	**EH**, SK, LN	**EH**, MI, LN
VI	**EH**	**EH,** KH, ML	**EH**	**EH**, KK, LN	**EH**, LN
VII	ML, SL, LN	ML, LN, **EH**	ML, LN, **EH**	ML, LN, **EH**	**EH**, AA, LN

Aro, R. − RA
Astover, A. − AA
Haiba, E. − EH
Herodes, K. − KH
Ivask, M. − MI
Järvis, J. − JJ
Kipper, K. − KK
Kutti, S. − SK
Kuu, A. − AK
Laaniste, A. − AL
Lillenberg, M. − ML
Litvin, S.V. − SL
Nei, L. − LN
Peda, J. − JP

OTHER PUBLICATIONS & CONFERENCE PRESENTATIONS

Haiba, E., Nei, L. (2017). Sewage Sludge Composting and Pharmaceuticals. −*7th International Scientific Forum, ISF 2017.* Proceedings. European Scientific Institute, Oxford, United Kingdom, February 7-9, 2017, pp 114−121.

Nei, L.*, **Haiba, E**., Lillenberg, M. (2016). Pharmaceuticals and Sewage Sludge Compost. Abstracts book: *International Congress on Water, Waste and Energy Management*, ScienceKNOW Conferences, Rome, Italy, July 18-20, 2016, pp 186−187.

Haiba, E.*, Nei, L. (2016). Composting studies in Estonia. Abstract book: *7th SETAC World Congress/SETAC North America 37th Annual Meeting*. Orlando, Florida, USA, November 6−10, 2016.

Haiba, E., Ivask, M., Olle, L., Peda, J., Kuu, A., Kutti, S., Nei, L. (2014). Transformation of nutrients and organic matter in vermicomposting of sewage sludge and kitchen wastes. *Journal of Agricultural Science*, 6 (2), pp 114−118.

Haiba, E.*, Kipper, K., Nei, L., Lillenberg, M., Herodes, K. (2013). Pharmaceuticals in Sewage Sludge Compost. *SETAC Europe 23rd Annual Meeting*, Glasgow, UK, May 12−16, 2013.

Haiba, E.*, Lillenberg, M. (2012). Degradation of pharmaceuticals in sewage sludge compost. Abstract book: *SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting*, Berlin, Germany, May 20−24, 2012.

Nei, L., Lillenberg, M., **Haiba, E**.* (2012). Plant uptake of some commonly used pharmaceuticals. Abstract book: *SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting*, Berlin, Germany, May 20−24, 2012.

Nei, L., Lillenberg, M., **Haiba, E**., Kipper, K., Herodes, K. (2011). Pharmaceuticals in Sewage Sludge Compost and Their Uptake from Fertilized Soil by Food Plants. Abstract Book: *Society of Environmental Toxicology and Chemistry North America 32nd Annual Meeting*. Boston, Massachusetts, USA, November 13−17, 2011, 241.

Kipper, K., Herodes, K., Lillenberg, M., Nei, L., **Haiba, E**., Litvin, S.V. (2010). Plant Uptake of some Pharmaceuticals Commonly Present in Sewage Sludge Compost. In: *Proceedings of 2nd International Conference on Chemical, Biological and Environmental Engineering (ICBEE)*, 261−264.

* *presented at the conference*

ABBREVIATIONS

AM	antimicrobials
C	carbon
CBZ	carbamazepine
CIP	ciprofloxacin
DCF	diclofenac
DNA	deoxyribonucleic acid
dw	dry weight
EU	European Union
FQs	fluoroquinolones
GUA	guanylurea
HESI	heated electrospray interface
K_{biol}	biological degradation rate constant
K_d	sorption coefficient
K_{ow}	octanol-water partition coefficient
LC-ESI-MS	liquid chromatography electrospray ionization – mass spectrometry
LC-MS	liquid-chromatography – mass spectrometry
MET	metformin
MRM	multiple reaction monitoring
N	nitrogen
NOR	norfloxacin
NSAID	non-steroidal anti-inflammatory drug
OFL	ofloxacin
PhACs	pharmaceutically active compounds
PLE	pressurized liquid extraction
PPCPs	pharmaceuticals and personal care products
RSD	relative standard deviation
SAs	sulfonamides
SDM	sulfadimethoxine
SIR	substrate induced respiration
SMX	sulfamethoxazole
SPE	solid phase extraction
STP	sewage treatment plant
$t_{1/2}$	half-life values
TCS	triclosan
v:v	volume to volume
VAs	veterinary antibiotics
WWTPs	wastewater treatment plants

INTRODUCTION

The amount of sewage sludge generated by mankind is increasing rapidly all over the world (White et al., 2011). Intelligent disposal of this matter is one of the major environmental tasks that needs to be solved (Nafez et al., 2015), strictly keeping safety in mind. In spite of the fact that sewage sludge and sewage sludge compost both contain a large number of different pollutants, involving residues of pharmaceuticals (Lillenberg et al., 2009; Lillenberg et al., 2010a; Haiba et al., 2013a, b), their usage is increasingly popular in agriculture (Noirot-Cosson et al., 2016), forestry (Järvis et al., 2016), horticulture (De Lucia et al., 2013), and in restoration of abandoned mining areas (Varnik et al., 2006).

Crop production in post-soviet countries like Estonia largely takes place at the expense of soil phosphorous resources (Astover and Rossner, 2013; Haiba et al., 2016). Sewage sludge compost is without a doubt an important source of nutrients, but at the same time it may perform in the role of hazardous waste, associated with several severe phenomena such as soil and plant pollution or microbial resistance.

Pharmaceutical products are being increasingly detected in the environment (Amouzgar and Salamatinia, 2015). The presence of pharmaceutical residues (and even in very low concentrations) in sewage sludge compost is of great concern (Haiba et al., 2017). 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, an 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 and Mazel, 2011).

Recent decades have shown intensive work in studying the fate of pharmaceuticals in the environment originating from sewage treatment plants (STPs). These studies involve the development of analytical tools (Lillenberg et al., 2009; Kipper et al., 2011; Haiba et al., 2017), determination of pharmaceuticals in different compartments (Lillenberg et al., 2010a), composting technologies (Haiba et al., 2013b) and plant uptake of pharmaceuticals (Lillenberg et al., 2010b; Eggen et al., 2011; Colon and Toor, 2016). Pharmaceuticals entering into the soil may affect microbial activity, plant growth and development, and may have adverse effects on living organisms (Haiba et al., 2017). A number of pharmaceuticals, known to be persistent in soil, are able to accumulate into food plants (Jjemba, 2002; Migliore et al., 2003; Boxall et al., 2006; Dolliver et al., 2007; Haiba et al., 2013a). The fate of human pharmaceuticals in the environment is presented in figure 1.1.

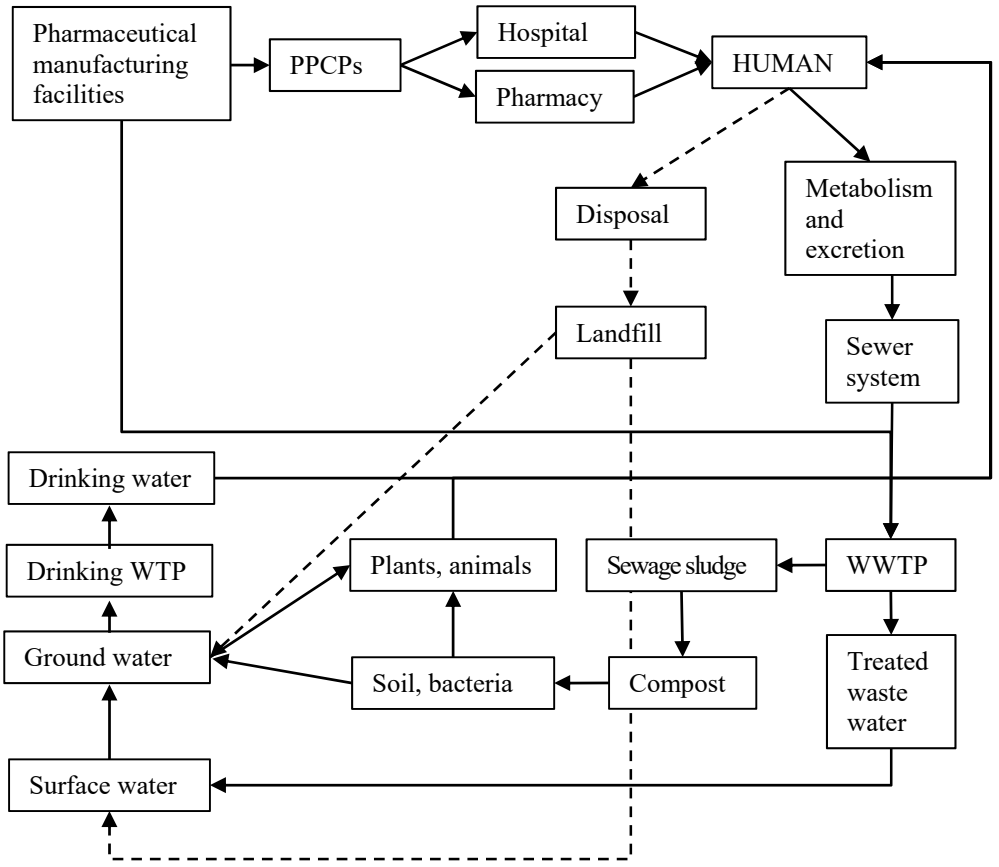

Figure 1. Fate of human pharmaceuticals in the environment

It would be impossible to determine the presence and content of all possible pharmaceuticals in compost and to study their uptake by different food crops. This leads to the understanding that compost must be made safe using reliable sewage sludge treatment technologies, based on research. The safety of using compost should be ensured using universal testing methodologies developed for this purpose. Recent research shows that pharmaceuticals and household products can be degraded during composting (Patureau et al., 2008; Poulsen and Bester, 2010; Zhang et al., 2011). However, the literature data on this topic are scarce and more knowledge is required in this field (Butovskyi et al., 2016). It has been shown that by using different amendments, the effectiveness of the degradation of pharmaceuticals may increase during composting (Qiu et al., 2012).

The current thesis is to summarise the work published in the papers listed under the heading "List of original publications that constitute the thesis".

1. LITERATURE REVIEW

1.1 Sewage-derived pharmaceuticals in the environment (I, II, III, IV, V, VI, VII)

Pharmaceuticals have been used for decades to prevent and treat human and animal diseases (Zhang et al., 2008; Li et al., 2014; Haiba et al., 2016). Over the past 15 years, the adverse effects (including endocrine disruption and antibiotic resistance) of some compounds, called “emerging”, have been observed in animals, humans and other organisms (Peysson and Vulliet, 2013). Several studies have demonstrated that the two most important sources through which pharmaceuticals reach the environment are sewage sludge and its compost (Kim et al., 2012; Rodríguez-Rodríguez et al.,2012; Reichel et al., 2013, Jelic et al., 2011, Borgman and Chefetz, 2013; Haiba et al., 2016; Kipper et al., 2017).

The problems of handling sewage sludge and the reduction of its environmental impacts has become important in the whole world. The most common and cost-effective use of sludge, compared to the other methods such as incineration, has become agricultural application (Zuloaga et al., 2012; Li et al., 2013a; Chen et al., 2014; Haiba et al., 2016). The use of sewage sludge in agriculture is one of the major causes of environmental pollution (Nouri et al., 2008). Though sewage sludge and its compost offers an opportunity to recycle plant nutrients and organic matter to soil for crop production stimulating biological activity (Rodríguez et al., 2012; Zuloaga et al., 2012; Li et al., 2013a; Haiba et al., 2014), its usage as a fertilizer is limited due to a large number of toxic pollutants found in this matter (Lillenberg et al., 2010a; Lillenberg, 2011; Haiba et al., 2016).

Medications used for human medical care, such as analgesics, antibiotics, anti-inflammatories, antidepressants and antiepileptic drugs do not decompose completely in the human body (Bergersen et al., 2012, Zhang et al., 2008; Vasskog et al., 2009). The major route by which pharmaceuticals enter sewage is commonly accepted to be via urine and faces, with each contributing different relative amounts depending on the pharmacokinetics and structure of the individual compound (Winkler et al., 2008; Haiba and Nei 2017). A range of studies has shown that some pharmaceuticals and personal care products (PPCPs) are neither completely removed by sewage treatment, nor completely degraded in the environment (Redshaw et al., 2008; Lillenberg et al., 2009; Lillenberg et al., 2010a; Jelic et al., 2011; Rodríguez-Rodríguez et al., 2012; Borgman and Chefetz, 2013; Haiba et al., 2013b; Narumiya et al., 2013; Reichel et al., 2013; Haiba et al., 2016). Large amounts of PPCP residues have been found in terrestrial environments where soil has been fertilized with sewage sludge compost (Ho et al., 2013; Peysson and Vulliet, 2013; McClellan and Halden, 2010).

Antibiotics are designed to be subject to biodegradation and "work" effectively even at small doses (Girardi et al., 2011). Pharmaceuticals can affect the efficiency of microbial-mediated processes (the regeneration of nutrients, carbon and nitrogen circulation and digestion of pollutants) in the environment (Girardi

et al., 2011; Jelic et al., 2011; Bergersen et al., 2012; Martín et al., 2012a; Chen et al., 2013; Li et al., 2014; Haiba et al., 2016).

As a result of regular industrial, agricultural and household activities, a variety of compounds enter into the environment, of which only a small percentage are studied for their toxicological effects on humans and the environment (Peysson and Vulliet, 2013). Approximately 4000 drug substances are used in Europe (human and veterinary) and they are susceptible to reach the environment (Mompelat et al., 2009; Rodríguez-Rodríguez et al., 2011). Scientists have studied about 150 medical compounds that have been found in the environment, mostly in water samples (Rivera-Utrilla et al., 2013; Li et al., 2014). When drugs are detected in the environment, their concentrations are generally in the ng/L-μg/L (ppt-ppb) range (Moldovan et al., 2009; Haiba and Nei, 2017). Individual concentrations of any drug might be very low, but the combined concentrations from drugs sharing a common mechanism of action could be substantial (Daughton and Ternes, 1999; Haiba and Nei, 2017).

There are increasing concerns about the undesired impacts that may result from continuous contamination of the environment with pharmaceutically-active substances (Barbosa et al., 2016; Verlicchi and Zambello, 2016). One of the possible fates of pharmaceuticals is to accumulate in organisms. Bioaccumulation may have different effects, from increased internal loads in a given organism potentially reaching toxic concentrations to biomagnification through up-concentration along a food chain (Straub, 2016; Haiba and Nei, 2017).

Antibiotics present in soil contaminated with pharmaceutical residues may be taken up by plants from arable land or pasture, and thus involuntarily end up in human or animal food, destroy soil microorganisms or develop drug resistance. Genes determining drug resistance can be transferred from harmless soil microbes to pathogenic microbes (Davies, 1994; Haiba and Nei 2017). It is assumed that using sewage sludge or manure containing drug residues for fertilizing is one of the main reasons of increasing drug resistance (Knapp et al., 2010; Haiba and Nei 2017).

Medical substances have many necessary properties to bio-accumulate and provoke change in ecosystems (Kipper et al., 2010; Baran et al., 2011). The regulatory acts lack the trigger values for pharmaceuticals in sewage sludge (Decree of Estonian Minister of the Environment; EU Council Directive 86/278/EEC; Lillenberg et al., 2009). The most closely related act is the European Union (EU) directive EMEA/CVMP/055/96 defining the quality of manure. The concentrations of pharmaceuticals must be under 100 μg kg^{-1} in manure and below 10 μg kg^{-1} in soil. Montforts (2005) suggests that these figures should be remarkably lower. Soil organisms, microflora and plants are directly exposed to contaminants in sludge-amended soils (Haiba et al., 2016). There have been many studies reflecting the occurrence and degradation of different pharmaceuticals in sewage sludge and its compost (Jelic et al., 2011; Nei et al, 2014; Lillenberg et al., 2010 a, b; Haiba et al., 2013 a, b; Kipper et al., 2010), but currently the knowledge of PPCPs ecotoxicology and their potential risks to the environment is weak and needs to be analysed (Li et al., 2014).

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 (Haiba et al., 2017). The development of new analytical methodologies is time-consuming and requires sufficient level of resources.

Numerous attempts have been made to create models directed to the estimation of potential concentrations of trace organic compounds in sewage sludge and biosolids (Gielen, 2007; Bock et al., 2010; Cunningham et al., 2011; Zhang et al. 2016). Unfortunately no unified approach is still available due to the complexity of interactions involving these compounds and sewage sludge.

1.2 Sewage sludge treatment and degradation of pharmaceuticals (I, III, IV, V)

Unprecedented growth in urban population has resulted in the generation of huge quantities of wastewater worldwide (Singh and Agrawal, 2010). Wastewater treatment facilities are responsible for treating large volumes of domestic and industrial sewage containing human waste. The goal of this activity is to produce effluents of high enough quality for discharge back into the environment. Sewage sludge is a byproduct of this process and necessitates proper disposal (Walters et al., 2010; Zuloaga et al., 2012; Haiba et al., 2016).

Historically, sewage sludge has been disposed of by landfilling, incineration or sea disposal (Hara and Mino, 2008; Bridle and Skrypski-Mantele, 2000). Nowadays, the most widespread method for sewage sludge disposal has become agricultural application, since it is the most economical outlet for sludge compared to landfilling and incineration (Zuloaga et al., 2012; Li et al., 2013a; Chen et al., 2014; Haiba et al., 2016). Sewage sludge compost is rich in nutrients and trace elements and could be re-used in agriculture as soil fertilizer stimulating its biological activity (Margesin et al., 2006). Nowadays more than 60% of the sewage sludge produced in the United States and 40% generated in Europe are applied to the land (Harrison et al., 2006; Zuloaga et al., 2012; Nei et al., 2014).

Sewage sludge may be regarded as hazardous waste, but it can also be used as a fertilizer. Its safety with respect to pharmaceutical residues must be assessed before use (Kipper et al., 2011). According to Nayak and Kalamdhad (2015) composting is one of the sustainable practices to convert sewage sludge into useful agricultural product. Alternatively, it has been stated that sewage compost cannot be used for agricultural purposes: it may contain an excess amount of chemical contaminants that can be assimilated by food crops (Lillenberg et al., 2010a). However, sewage compost is rich in minerals, enabling long-lasting supply for the fast growth of plants (Järvis et al., 2016; Haiba and Nei, 2017).

Sewage sludge is an inevitable by-product of wastewater treatment. For example, in Estonia about 360,000 − 500,000 tons of it is created annually. Sewage sludge, which is difficult to market, piles up at wastewater treatment

plants. Many pollutants are not efficiently removed during sewage and sewage sludge treatment (Martín et al., 2015; Haiba and Nei, 2017). 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. Therefore, it is necessary to stabilize the organic residues in sewage sludge by composting (Knepper and Barceló, 2003; Oleszczuk, 2009; Zuloaga et al., 2012). Figure 1.1. is to present the compost piles at Tallinn WWTP.

Pharmaceuticals can be degraded during composting (Poulsen and 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; Haiba et al., 2017).

Figure 1.1. Compost piles at Tallinn WWTP

Since sewage sludge has high moisture content it cannot be composted alone − in order to absorb moisture it should be mixed with dry materials, which act as bulking agents thereby improving the aeration and the compost quality (Nayak and Kalamdhad, 2015; Zhou et al., 2014; Haiba et al., 2016). Sludge and bulking agent proportions in compost influence the composting reaction rate and the final compost quality. Onwosi et al. (2017) reported that C and N are the most crucial nutrients needed by the microorganisms involved in composting: C is used as energy source while N is used for building cell structure (Chen et al., 2011a; Iqbal et al., 2015). Therefore, the C/N ratio is an indicator of the degree of decomposition of an organic matter, as C is lost as CO_2 during bio-oxidation (Lazcano et al., 2008; Onwosi et al., 2017). Sludge can be mixed with different bulking agents, sources of carbon, such as peat, straw, wood chips, leaves, peat, rice husk, peanut shells and sawdust (Komilis et al., 2011; Cukjati et al., 2012; Maulini-Duran et al., 2013; Malinska et al., 2014; Haiba et al., 2016; Onwosi et al., 2017).

The degradation rate of pharmaceutical residues is dependent on the initial components of the compost. Only very few o publications are dealing with the impact of the composition of compost on the degradation of pharmaceuticals. Hardwood sawdust appears to be an excellent sewage sludge amendment: from the agricultural point of view, sludge co-composted with particularly fine-textured sawdust is claimed to be an excellent compost material to be applied to soils (Ammari et al., 2012; Nei et al., 2015, Haiba et al., 2016). Kim et al. (2012) have shown that sawdust is able to initiate efficient composting, leading to elevated composting temperatures, and consequently resulting in the reduction of residual concentrations of pharmaceuticals to reasonable levels in a relatively short composting period (Haiba et al., 2016). The degradation of salinomycin was observed by Ramaswamy et al. (2010) under open and composting conditions.

Butkovskyi et al. (2016) studied the degradation of pharmaceuticals during composting of the excess sludge from UASB (Upflow Anaerobic Sludge Bed) with waste wood under controlled conditions. The results showed that the degradation ranged from 87.8% for carbamazepine to 99.9% for estrone, diclofenac and ibuprofen. According to Lillenberg (2011) the degradation of pharmaceutical residues was more efficient in compost when anaerobically digested sludge was mixed with peat, compared to the results when raw sewage sludge was mixed with tree bark (Haiba et al., 2016).

Co-composting manure with sawdust or rice straw has shown more effective degradation rates for sulfonamides (SAs) than treatments using manure alone (Qiu et al., 2012). Kim et al. (2012) indicated that well organised composting process resulting in an efficient decline of residual veterinary antibiotics (VAs) originating from livestock manures will require some source of organic matter, as the organic matter can elevate temperatures and provide a wide range of additional binding sites during composting. Moreover this study recommended that application of livestock manure as raw material and/or as liquid fertilizer after only a short storage period to stabilize the manure should be avoided as this may result in the potential release of VAs to the environment (Kim et al., 2012).

The results involving the evaluation of the biological degradation and sorption of carbamazepine (CBZ), diclofenac (DCF) and some other pharmaceuticals during the secondary treatment in WWTPs activated sludge were published by Martínez-Alcalá et al. in 2017. The biological degradation rate constant (K_{biol}) is estimated to follow a pseudo first-order equation (Joss et al., 2006a):

$$\frac{dS_t}{dt} = K_{biol} \times MLSS \times S_t, \quad (1)$$

where S_t is the soluble compound concentration at time t (ng L^{-1}), t is hydraulic retention time (h), K_{biol} is the intrinsic biological rate constant (L g^{-1}_{ss} h^{-1}), *MLSS* is the suspended solids average concentration (g L^{-1}).

The equation allows predicting the elimination rate of pharmaceuticals. In WWTPs with conventional activated sludge systems, the value of K_{biol} allows the

formation of three groups of substances (Martínez-Alcalá et al., 2017; Joss et al., 2006a):

a) Substances with $K_{biol} < 0.1$ do not degrade in a significant grade (<20%);
b) Substances with $0.1 < K_{biol} < 10$ show a partial degradation (between 20% and 90%);
c) Substances with $K_{biol} > 10$ perform a high degradation (>90%).

The sorption coefficient (K_d) of the pharmaceutically active compounds (PhACs) is defined for equilibrium conditions (Martínez-Alcalá et al., 2017; Nielsen and Bandosz, 2016; Joss et al., 2006a). The following equation is used to express the extent of sorption:

$$K_d = \frac{X}{MLSS \times S}, \tag{2}$$

where K_d is the sorption coefficient of activated sludge (L g^{-1}_{ss}), X is the sorbed compound concentration in ng L^{-1}, $MLSS$ is the suspended solids concentration (kg L^{-1}) and S is the soluble compound concentration (ng L^{-1}).

The study conducted by Martínez-Alcalá et al. (2017) showed that in removing CBZ and DCF from wastewater in WWTP, the sorption of these compounds clearly dominated over microorganism degradation. According to the classification given by Joss et al. (2006a), the removal of CBZ does not take place by biodegradation ($K_{biol} = -0.87$ L g^{-1}_{ss} h^{-1}), whereas in the case of DCF partial biodegradation can be followed ($K_{biol} = 1.31$ L g^{-1}_{ss} h^{-1}). At the same time, the elimination of CBZ via sorption is higher ($K_d = 0.47$ L g^{-1}_{ss}). Although the sorption coefficient for DCF is lower (0.11 L g^{-1}_{ss}), it is still high enough if compared to other pharmaceuticals studied by Martínez-Alcalá et al.

For triclosan (TCS), K_{biol} = (0.05…0.15) L g^{-1}_{ss} h^{-1} and $K_d = 2.5$ L g^{-1}_{ss}, and for metformin (MET) the relevant values are (0.30…0.54) L g^{-1}_{ss} h^{-1} and 0.03 L g^{-1}_{ss}, showing that the removal of TCS mainly takes place via sorption, whereas the removal of MET realises through biodegradation (Blair et al., 2015).

The removal of the target pharmaceuticals during composting can be expressed as first-order kinetics (Ho et al., 2013):

$$C = C_0 e^{-kt}, \tag{3}$$

where C is the analyte concentration (mg/kg) at time t (day), C_0 is the initial analyte concentration (mg/kg) and k is the antibiotic removal rate constant (day^{-1}). The half-life ($t_{1/2}$) of the analytes can be calculated as follows:

$$t_{1/2} = -ln2/k \tag{4}$$

With the aim of calculating the expected levels of pharmaceuticals in WWTP, the drugs consumption data together with reporter values for the percentage of pharmaceuticals excreted, removal in WWTP and partitioning into sludge can be applied in relevance to the methodology described by Gielen (2007), Khan and Ongerth (2004) and Castiglioni et al. (2004). The concentrations of pharmaceuticals in sewage influent (C_{inf} [ng/L]) were calculated using the following equation:

$$C_{inf} = \frac{M \times P_{STP} \times 10^9}{Fr \times P_{survey}}, \quad (5)$$

where M is the mass of excreted pharmaceutical (kg), P_{STP} the population served by the sewage treatment plant, P_{survey} the population contributing to consumption survey, Fr the annual sewage influent flow rate (m^3).

The expected concentrations of pharmaceuticals in sewage effluent C_{eff} (ng/L) can be calculated using equation 5, where M_{ST} is the mass of pharmaceutical in effluent after sewage treatment in WWTP:

$$C_{eff} = \frac{M_{ST} \times P_{STP} \times 10^9}{Fr \times P_{survey}}, \quad (6)$$

The concentrations of pharmaceuticals in sewage sludge C_{SS} [ng/g] can be calculated using the partitioning percentages (Gielen, 2007; Khan and Ongerth, 2004) and modification to equation 5.

$$C_{SS} = \frac{M_p \times P_{STP} \times 10^9}{SS \times (1-mc) \times P_{survey}}, \quad (7)$$

where M_p is the amount of pharmaceutical partitioned into the sewage sludge phase (kg), P_{stp} the population served by the sewage treatment plant, SS is the annual production of sewage sludge (kg), mc the moisture content of the sewage sludge and P_{survey} the population contributing to consumption survey.

1.3 Pharmaceuticals used in the current study

Several experiments were conducted with fluoroquinolones and sulphonamides with the aim of selecting the most appropriate composting technology that would be available under real conditions in the countries with similar climate to Estonia. The usage and properties of these pharmaceuticals are described in the dissertations of Merike Lillenberg (Lillenberg, 2011) and Karin Kipper (Kipper, 2012). The current work also involves the studies with diclofenac, carbamazepine, metformin and triclosan. The first of them confirmed the occurrence of considerable concentrations of pharmaceuticals in sewage sludge, and the work completed by Karin Kipper concentrated on the development of novel reliable methodologies for the determination of pharmaceuticals in different media.

1.3.1 Fluoroquinolones

The fluoroquinolones (FQs) used in the current study were ciprofloxacin (CIP), norfloxacin (NOR) and ofloxacin (OFL). The information concerning their structure, properties and usage can be found from the following sources: Schaumann and Rodloff, 2007; Lillenberg, 2011; Doorslaer et al., 2014; Merck Manuals[1]; RxList[1]; RxList[2]; RxList[3].

1.3.2 Sulfonamides

The following sulphonamides (SAs) were used in the current study: sulfamethoxazole (SMX) and sulfadimethoxine (SDM). The information concerning their structure, properties and usage can be found from the following sources: Drugbank; Merck Manuals[2]; Merck Manuals[3]; Richardson and Bowron, 1985; Carballa et al., 2004; Ingerslev and Halling-Sørensen, 2000; Pérez et al., 2005; Hamscher, 2002; Batt et al., 2007; Avisar *et al.*, 2010; Li and Zhang, 2010; Lillenberg, 2011.

1.3.3 Diclofenac

Diclofenac (DCF) is one of the most popular non-prescription medicals (Haiba et al., 2017). It is non-steroidal anti-inflammatory drug (NSAID) and widely used for relieving pain (Chen et al., 2015). DCF together with its human metabolites enter 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). DCF residues have been detected in sewage sludge with concentrations reported from 2 ng g^{-1} to 140 ng g^{-1} respectively (Jelić et al., 2009; Dobor et al., 2010; Jelić et al., 2011; Loos et al., 2013). DCF residues have been detected in aqueous environments (Al-Rajab et al., 2010) where they can cause deoxyribonucleic acid (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 DCF has been studied and described in mammals, fungi and microorganisms (Huber et al., 2012; Bartha et al., 2014). DCF is acutely toxic to birds and presumably could leach into soil beneath the corpses of livestock containing diclofenac residues (Oaks et al., 2004; Stülten et al., 2008; Al-Rajab et al., 2010; Haiba et al., 2017).

1.3.4 Carbamazepine

Carbamazepine (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 faeces (RxList[4]; Zhang et al., 2008). Nieto et al. (2010) determined concentrations between 11 and 42 mg/kg (dry weight - dw) for CBZ in samples from two STPs. However Miao et al. (2005) detected CBZ at concentration 69.6 µg/kg (dry weight) in untreated biosolids and at concentration 258.1 µg/kg (dry weight) in treated biosolids. It has been indicated that CBZ exhibits the persistence characteristic of organic contaminants, potentially leading to long-term environmental risks (Chefetz et al., 2008). 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).

Antiepileptic drug 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., 2006b; Verlicchi et al., 2012). Removal of CBZ and its metabolites from municipal sewage treatment plant is very low (~8%). In some cases CBZ exhibits even negative removal efficiency (Collado et al., 2014) with no seasonal variation (Golovko et al., 2014). CBZ is persistent in soils (Paltiel et al., 2016; Li et al., 2013b; Grossberger et al., 2014) and has been shown to be taken up and accumulate in a variety of crops (Malchi et al., 2014, Goldstein et al., 2014; Holling et al., 2012; Winker et al., 2010; Shenker et al., 2011).

CBZ is recalcitrant both in biodegradation and photolysis experiments. CBZ 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-Álvareza et al., 2015). Researchers have quantified acute toxicity of CBZ <100 mg/L (Malarvizhi et al., 2012).

Compounds with log K_{ow} < 2.5 (K_{ow} is octanol-water partition coefficient) are assumed to have a low potential for adsorption onto particulates (Hawker and Connell 1988). The metabolites of CBZ have logK_{ow} values between 0.13 and 2.41, in comparison to the log K_{ow} for CBZ of 2.67. The concentrations of CBZ and metabolites increased on a dry weight (dw) basis between untreated and treated biosolids (Miao et al., 2005).

1.3.5 Metformin

Metformin (MET) is the first-line medication for the treatment of type 2 diabetes (Maruthur et al., 2016), particularly in people who are overweight (Tsigos et al., 2008). This disease affects more than 200 million people worldwide (Reitman and Schadt, 2007; Trautwein and Kümmerer, 2011). The results published in 2015 by Niemuth and Klaper demonstrated that MET acts as an endocrine disruptor at environmentally relevant concentrations.

MET was present in every water sample tested by Kümmerer's team, including even tap water. Kümmerer and his co-authors concluded that the drug is likely "distributed over a large fraction of the world's potable water sources and ocean" (Trautwein et al., 2014). Unlike many pharmaceutical drugs, MET is not metabolized by humans but passes unchanged through the body. Entering aquatic compartments, such as in sewage, it can be transformed bacterially to the ultimate transformation product Guanylurea (GUA). With no natural degradation processes, both these compounds can be easily reintroduced to humans as they enter the food chain (Trautwein et al., 2014). Detection of MET and GUA 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).

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 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 and Stefańska, 2014). This indicates that this drug (or its metabolite – GUA) may be a potential threat to ground and surface water (Benotti and Brownawell, 2005, 2008). Half-life values ($t_{1/2}$) for aerobic conditions were from 1 to 5 days depending on soil type.

1.3.6 Triclosan

Triclosan (TCS) is a broad-spectrum antimicrobial compound, commonly used in personal care products such as soaps, creams, toothpastes and detergents, and in housewares (cutting boards, even textiles and toys). TCS has been used for over 40 years. The use of antimicrobials (AM) and antibacterial products is increasing all over the world (Haiba et al., 2017; Lozano et al., 2010). Today, TCS compounds are consumed in Europe at approximately 350 tons per year (Pintado-Herrera et al., 2014). TCS residues have been detected in wastewater (in concentrations ranging from 1-10 $\mu g L^{-1}$) as well as in sewage sludge (concentration range 2-8 mg kg^{-1} dry matter) (Chen et al., 2011b; Loos et al., 2013). TCS residues have been found in soils fertilized with sewage sludge compost up to a concentration of 4 $\mu g\ kg^{-1}$. Various studies have shown that already at relatively low concentrations TSC may have adverse effects to the environment – preventing bacterial metabolism, affecting microbial respiratory activity and populations (Lozano et al., 2010; Chen, et al., 2011b; Pintado-Herrera et al., 2014; Haiba et al., 2017).

2. AIMS OF THE STUDY

Composting sewage sludge is a good way to recycle this nutrient-rich material. On the one hand, the amount of generated waste would be reduced and, on the other hand, cheap fertilizers used in agriculture would be produced. Untreated sewage sludge may contain heavy metals, pathogens and PPCPs that may not be destroyed during wastewater treatment processes. Therefore it is important to find ways to make the degradation on PPCPs more efficient in sewage sludge composting process. At the moment, there is little information how long-term use of sewage sludge and contaminants present therein affects the environment, humans and animals, and this area should definitely be explored.

The aim of this work was to study the impact of sewage sludge composting on the degradation of some widely used pharmaceuticals and to add a piece of knowledge applicable in the development of composting technologies leading to the increase of the safety of using sewage sludge compost in fertilization of soils with poor nutrient content. Plant uptake experiments of pharmaceuticals were to confirm the importance of the present study. The body of the current thesis is summarising the work published in the papers listed under the heading "List of publications".

The main directions of the research presented in this thesis were:

- To study the possibility of reducing the impact of some widely used pharmaceuticals on the environment and people through increasing the efficiency of sewage sludge composting technologies.
- To develop efficient sewage sludge composting conditions with the aim of achieving more complete degradation of FQ and SA residues.
- To determine the impact of bulking agent on the degradation of DCF, MET, CBZ and TCS residues in sewage sludge compost.
- To give recommendations concerning sewage sludge treatment.

3. MATERIALS AND METHODS (I, II, III, V, VI, VII)

The methodologies of chemical analysis presented below were developed and the determinations of the concentrations of pharmaceuticals were carried out at the University of Tartu, Institute of Chemistry. The experimental details with fluoroquinolones and sulphonamides are presented in detail in Lillenberg, 2011; Kipper, 2012; Haiba et al., 2013a; Haiba et al., 2013b and Kipper et al, 2017. The selection of AM was made according to preliminary pilot study on antimicrobials usage and presence in the sewage sludge samples (Lillenberg et al., 2009; Lillenberg, 2011), their stability in the soil and potential degradation during the composting procedure (Lillenberg et al., 2010a) and their uptake from the soil by plants (Eggen et al., 2011; Michelini et al., 2012). The experimental details with diclofenac, metformin, carbamazepine and triclosan are partly presented in detail in Haiba et al., 2017 and in Haiba and Nei, 2017.

3.1 Composting experiments with fluoroquinolones and sulphonamides

3.1.1 Experiments with sewage sludge and compost

The collection and treatment of sewage sludge and compost samples, the methodology used for the determination of AM from sewage sludge and compost together with method validation are described in detail in Kipper, 2012; Lillenberg, 2011; and in 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%.

Model experiments with different compost mixtures were performed with the aim of establishing the impact of the compost composition on the degradation of some pharmaceuticals of FQs and SAs. Method for simultaneous determination of CIP, NOR, OFL, SDM and SMX from sewage sludge compost consisted of 3 parts: pressurized liquid extraction (PLE), solid phase extraction (SPE), and liquid-chromatography - mass spectrometry (LC-MS). The methodology used for the determination of antimicrobials from sewage sludge compost was based on the methodology described in Lillenberg et al. (2009).

3.1.2 Plant uptake experiments

Plant uptake experiments were aimed to show the importance of keeping the content of pharmaceutical residues in sewage sludge compost under control. These experiments are presented in detail in the following publications: Lillenberg, 2011; Kipper, 2012; Haiba et al., 2013a and Kipper et al., 2017. Plant uptake experiments were carried out with potatoes (*Solanum tuberosum L*), carrots (*Daucus carota L*), lettuce (*Lactuca sativa L*) and wheat (*Triticum vulgare*

L). The pharmaceuticals used were CIP, NOR, OFL, SDM, and SMX. Three parallel experiments were conducted for each concentration of antimicrobials. In reference experiments, plants were cultivated in antimicrobial-free soils. The plants were collected and washed carefully. Potatoes and carrots were chopped into ca 1 cm^3 pieces. Then the plants were dried at room temperature in the darkness and after that milled for analyses, using Knifetec 1095 Sample Mill (Foss) and a common coffee mill. The size of the particles of the powder was < 1 mm^3. The milled samples were dewatered in a thermostat at 45°C for 24 hours using thermostat Binder KB 115 and stored in hermetic plastic bags for three weeks at –80 °C (using refrigerator Sanyo MDF-U54V) before analysis.

3.1.3 Determination of antimicrobials from plants

The details of the methodology used for the determination of antimicrobials from plants are described in detail in the following publications: Lillenberg, 2011; Kipper, 2012; Haiba et al., 2013a and Kipper et al., 2017.

Chemicals. Pharmaceuticals were purchased from Riedel-de-Haën (Seelze, Germany) – three FQ-s: CIP (purity 99.8%), NOR (purity 99.9%) and OFL (purity 99.3%); and two SA-s: SDM (purity 99.4%) and SMX (purity 99.9%). Acetonitrile and methanol were obtained from J.T. Baker (Deventer, The Netherlands), HPLC grade formic acid and ammonia from Riedel-de-Haën. HFIP was purchased from Sigma (St. Louis, MO, USA). All solvents were of reagent grade or higher quality. Water was purified (18.2 MΩ×cm at 25 °C and a TOC value below 3 ppb) in-house using a Milli-Q Plus system from Millipore (Bedford, USA). Hydrophilic-lipophilic balanced (HLB) solid phase extraction (SPE) cartridges (Oasis HLB (60 μm), 500 mg/6 mL) were purchased from Waters (Milford, MA, USA).

Analytical work involved liquid extraction, solid phase extraction, liquid chromatography electrospray ionization – mass spectrometry (LC-ESI-MS) and method validation. Determination of antibiotic residues in plant material has been demonstrated in Kipper *et al.*, (2011).

3.2 Sewage sludge composting experiments with diclofenac, triclosan, carbamazepine and metformin

This section was conducted according to Haiba et al., 2017. DCF was added to the study given its widespread use in medicine and the detection in the environment, including sewage sludge and sewage, which may be used as a fertilizer or for irrigation of crops. CBZ was added to the study for its increasing use in medicine. MET was added to the study for its increased usage in Estonian medicine and the lack of general data on the possible occurrence and effects in environment. TCS was included in the study due to its expanding use in personal care products and household supplies. Selected PPCP residues have been found in treated sewage

sludge, compost, surface and ground water (Walter et al., 2010; Rivera-Utrilla et al., 2013; Luo et al., 2014; Rodríguez-Rodríguez et al., 2011).

3.2.1 Chemicals and materials

Standard substances of pharmaceuticals were obtained from Sigma-Aldrich: DFC sodium salt (99.9%), TCS (99.7%), CBZ (99.9%) and MET hydroxide (99.8%). As 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%; 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) (Haiba et al., 2017).

3.2.2 Sample collection

Sewage sludge samples were collected from municipal wastewater treatment plant (Figure 3.1). The sludge was anaerobically digested and dewatered by centrifugation. 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; Kim et al., 2012; Mollazadeh, 2014) and previous studies on sludge composting with different bulking agents (straw, sawdust, oil-shale ash, wood chips) (Haiba et al., 2013; Nei et al., 2014). The initial concentration of every pharmaceutical was 2 mg kg^{-1} in relation to dw. In addition to this, two reference piles (without additions of pharmaceuticals) were prepared (Haiba et al., 2017).

Figure 3.1. Tallinn WWTP

3.2.3 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 sample tube.
2. Vortex mixed for 30 s.
3. Sample tube was tightly capped and placed horizontally on a shaker (200 rpm) for 10 min.
4. 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 7830 rpm for 5 min.
7. 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 weighed. 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 1400 µl of MilliQ water were added in 1.5 ml Eppendorf tube. Automatic pipette was used for dosing, but all the solutions were weighed. The solutions were vortex-mixed and filtered through syringe filter. The first five drops of filtrate were discarded and the remaining (ca 1 ml) was collected into auto-sampler vial (2 ml glass vial) (Haiba et al., 2017).

3.2.4 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 (Haiba et al., 2017).

3.2.5 LC-MS/MS analysis

Sample extracts were analysed 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 3.1. (partly taken from Haiba et al., 2017).

Table 3.1. 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
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 3000 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 (Haiba et al., 2017).

3.2.6 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. Samples with added pharmaceuticals were prepared in duplicates and each mixture had reference sample without pharmaceuticals. The room temperature was 23–26 ºC. Compost samples were mixed 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 (Haiba et al., 2017).

3.2.7 Microbial indices of sewage sludge compost

Microbial Substrate Induced Respiration (SIR) was determined via the Oxitop® manometric system (Platen and Wirtz, 1999). 50 gram of compost mixture was amended with glucose and incubated in a closed vessel at 22 ºC in the dark for 24 hours. After the incubation the microbial biomass C was calculated. To determine the microbial to fungal ratio the selective inhibition technique was used. In order to assess the fungal biomass the samples were treated with cyxloheximide (12 mg g^{-1}) and glucose (5 mg g^{-1}), and for the determination of bacterial biomass the samples were treated with streptomycin (6 mg g^{-1}) and glucose (5 mg g^{-1}). The controls were treated with both inhibitors cyxloheximide (12 mg/g) and streptomycin (6 mg g^{-1}). All the samples were incubated in closed vessels at 22 ºC in the dark for 24 hours, after which the biomass C was calculated. All the microbiological analyses were conducted in Tartu College, Tallinn University of Technology (Nei et al., 2014; Haiba et al., 2017).

4. RESULTS AND DISCUSSION

4.1 Fluoroquinolones and sulfonamides in sewage sludge compost and their plant uptake (VII)

This paragraph reflecting the pilot studies of the presence of fluoroquinolones and sulfonamides in sewage sludge compost and their plant uptake is based on the following publications (tables, figures and data used and reprinted with the kind permission granted by African Journal of Agricultural Research):

Haiba, E., Lillenberg, M., Kipper, K., Astover, A., Herodes, K., Ivask, M., Kuu, A., Litvin, S.V., Nei, L. (2013a). Fluoroquinolones and sulfonamides in sewage sludge compost and their uptake from soil into food plants. African Journal of Agricultural Research, 8, 3000−3006.

This pilot study was to show that pharmaceuticals are commonly present in sewage sludge and in its compost and plant uptake of pharmaceuticals from the soil fertilised with compost cannot be ignored in the view of food safety. The concentrations of FQs (CIP, NOR and OFL) and SAs (SDM and SMX) in sewage sludge samples from two WWTPs (located in the cities of Tartu and Tallinn) were determined (table 4.1).

Table 4.1. The maximum concentrations of FQs and SAs found from two WWTPs sludge samples

Sample	Sewage sludge treatmet technology	Pharmaceutical concentration µg/kg (dw)				
		CIP	NOR	OFL	SDM	SMX
Tallinn	anaerobically digested	1520	580	134	73	22
Tartu	compressed by filtration	442	439	157	27	8

According to European Union Directive (EMEA/CVMP/055/96, 1998) the sum concentration of pharmaceuticals should not exceed 100 µg/kg in manure and 10 µg/kg in soil fertilized with manure (Lillenberg, 2011). For the prevention of the development of microbial resistance of humans and animals the concentration of pharmaceuticals should be clearly under 0.1 µg/kg in agricultural soil (Lillenberg, 2011). The limited selection of results given above clearly shows that raw sewage sludge is not suitable for fertilising agricultural soils (Haiba and Nei, 2017). In Tartu WWTP the studied pharmaceuticals were not completely degraded even during twelve months in the compost mixture. In some Tartu compost samples the concentrations of CIP, OFL and NOR sufficiently exceeded the threshold concentration – 1 µg/kg – for pharmaceuticals in soil, although the WWTP considered the compost being ready for commercialization. The values of the highest detected concentrations of these pharmaceuticals in compost were respectively 70, 64 and 8 µg/kg. The concentrations of OFL and SMX were

lower, but still exceeded 1 µg/kg. SDM was absent after the twelve–months composing period.

The results given in Haiba et al. (2013a) show, that the degradation of FQs and SAs takes place during composting. After twelve months from the starting point of the preparation of the compost mixture in Tallinn the concentrations of FQs and SAs were in most cases below the limit of detection. As a rule, due to climate conditions in Estonia (cold winters and freezing), the twelve–months composting period does not correspond to the desire of WWTPs to finalise the process faster. Unfortunately, the six–months composting period is not sufficient when current composting technologies are used (Lillenberg, 2011 and Haiba et al., 2013).

Despite mixing the compost mixtures are still heterogeneous. The concentrations of pharmaceuticals varied remarkably within the same compost stack. For example, the concentrations of FQs differed up to 1.8 times within the same stack in Tartu. This phenomenon can be explained by uneven distribution of pharmaceuticals as a result of adsorption to solid sludge and bulking agent particles (Carmosini and Lee, 2008).

Presumably the main reason for the decrease in pharmaceutical concentrations during composting is the applied sludge treatment technology. The decomposition of pharmaceuticals was faster in Tallinn. In Tartu the sewage sludge compost was prepared by mixing the raw sludge with tree bark; in Tallinn the methane fermentation and mixing with peat were applied. The compost stacks were mixed regularly in both cities for promoting the growth of aerobic bacteria. Mixing/turning exposes different parts of the stack to the light. As photodegradation is considered to be one of the reasons for decomposition of FQs (Hooper and Wolfson, 1991), the intensity of compost stack turning might also have an impact on the decomposition of FQs in compost.

Application of sewage sludge and its compost to soils can lead to the contamination of food plants by pharmaceutical products. The uptake of the studied FQs and SAs was demonstrated from two different soil types (sandy and loamy) into food plants such as potato (*Solanum tuberosum L*), carrot (*Daucus Carlota L*) and wheat (*Triticum vulgare L*). The concentrations of the studied pharmaceuticals were relatively low in most of the plant samples, if compared to soil concentrations, but in some samples their concentrations were still of considerable magnitude, especially in plants grown in sandy soil. The uptake of FQs and SAs by potato might pose health risk, as the detected levels of the studied pharmaceuticals in potato tubers were remarkably high, exceeding in some samples the maximum residue levels (MRL) allowed for food of animal origin. Detectable amounts of CIP, OFL and SDM were also present in wheat seeds, but their concentrations were below MRL. These results raise human health concerns of consuming plants grown on compost-amended soils.

Plants accumulated pharmaceuticals from soil even at soil concentration 10 µg/kg (CIP and OFL). The residues of pharmaceuticals 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 (above the ground or underground). As a rule, the

higher concentrations of pharmaceuticals were followed in the case of sandy soil experiments. In loamy soil the molecules of SAs and FQs attach to clay particles, reducing their uptake by plants.

SAs are both fairly water-soluble and polar (Thiele-Bruhn et al., 2004; Haiba et al., 2013a). The low adsorption of SAs on soil particles is known (Beausse, 2004) and due to this phenomenon they are "ready" to migrate into plants. Different behaviour is characteristic of FQs. It has been shown that more than 90% of applied CIP and OFL are 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 (Haiba et al., 2013a).

The uptake of the studied pharmaceuticals by the selected food crops was apparent. Due to the low adsorption of SAs on soil particles they are “free” to migrate into plants. An opposite behaviour is characteristic of FQs. Therefore the content of SAs in the plants was usually higher. Interestingly, the amounts of FQs going into potato do not depend much on soil type.

The application of sewage sludge compost as a fertilizer and the following uptake of pharmaceuticals by food plants may cause contamination of these plants. The uptake of FQs and especially SAs by plants might pose risk to human health, as the concentrations of the studied pharmaceuticals were of considerable level, if compared to their soil concentrations. Due to this it would be an important task to exclude the exposure of plants to pharmaceuticals. This can be achieved through their complete degradation before sewage sludge compost is applied onto the agricultural land.

4.2 Bulking agent selection and degradation of fluoroquinolones and sulfonamides (VI)

This paragraph is an overview of the pilot study involving the selection of an amendment to sewage sludge when making compost. The texts, tables and data presented here are taken/reprinted from the paper Haiba et al., 2013b with the kind permission obtained from the journal AWERProcedia Advances in Applied Sciences.

Small quantities of the studied pharmaceuticals were present in sewage sludge that was used for preparing the compost mixtures (table 4.2) used in our experiments. “Blind” determinations of pharmaceuticals from the studied mixtures showed that the background concentrations of fluoroquinolones were never equal to zero (Table 4.3). This is in agreement with the results of the pilot study concerning the presence of pharmaceuticals in Tallinn and Tartu sewage sludge (section 4.1.).

Table 4.2. The composition of compost mixtures

Pile No	Sewage sludge treatment technology	Bulking agent (% from dry matter)	Dry matter, %
1	methane fermentation	peat (50)	23.1
2	methane fermentation + vermicomposting*	sawdust (33)	24.7
3	methane fermentation	sawdust + oil-shale ash (29+14)	32.3
4	compressed by centrifugation	sawdust + wood chips (total 43)	25.8
5	compressed by filtration	straw (50)	13.9
6	compressed by filtration + vermicomposting*	sawdust (33)	21.4
7	compressed by filtration	sawdust + oil-shale ash (29+14)	35.5

**Dendrobaena veneta* were added

The composting piles were turned periodically every 5-7 days for 4 months to maintain adequate oxygen levels and to homogenize the compost mixtures. After adding the pharmaceuticals to the sewage sludge and bulking agent mixtures their initial concentrations in dry matter were determined again. The results are presented in table 4.3. In most cases the concentration of each pharmaceutical was below 2 mg/kg. This was probably due to the phenomenon that the degradation of pharmaceuticals starts immediately after adding them to the compost mixture. Still, some of the concentrations (in table 4.3) are above this value probably due to the rapid sorption of pharmaceuticals (from liquid phase) to solid particles of sewage sludge or bulking agent. This is in agreement with the data presented in earlier studies (Golet et al., 2003; Göbel et al., 2005; Yang et al., 2011). After 4 months of composting, the concentrations of the formerly added pharmaceuticals were analytically determined again.

According to the data presented in table 4.4 it is evident that the degradation of pharmaceuticals was more complete when sawdust was used as a bulking agent (the degree of degradation of the total amount of pharmaceuticals was 94–98%), if compared to the sewage sludge mixtures with peat and straw (with 88% indicating the extent of degradation). There is no clear evidence that the addition of oil-shale ash influenced the degradation rate of the studied pharmaceuticals. As huge amounts of coal and oil-shale ash are produced every year and these wastes perform several good qualities as co-composting materials with sewage sludge, it would be reasonable to direct further studies on the establishment of the optimum composition of the sewage sludge compost with sawdust and oil-shale ash as co-composting agents.

Table 4.3. Degradation of pharmaceuticals in different compost samples

Pile No	Sample	Pharmaceuticals in dry weight, mg/kg				
		SMX	SDM	NOR	CIP	OFL
1	fresh	0.00	0.00	0.04	0.03	0.01
	fresh, with pharmaceuticals	2.23	1.78	1.52	0.98	1.68
	after 4-months composting	0.38	0.42	0.16	0.08	0.01
2	fresh	0.00	0.00	0.03	0.00	0.00
	fresh, with pharmaceuticals	2.04	2.22	2.00	1.35	1.89
	after 4-months composting	0.01	0.02	0.09	0.06	0.01
3	fresh	0.00	0.00	0.03	0.03	0.03
	fresh, with pharmaceuticals	1.74	1.78	1.72	1.11	1.54
	after 4-months composting	0.00	0.07	0.32	0.07	0.02
4	fresh	0.00	0.00	0.15	0.17	0.05
	fresh, with pharmaceuticals	2.11	1.37	2.33	2.31	3.12
	after 4-months composting	0.01	0.02	0.21	0.04	0.43
5	fresh	0.00	0.03	0.29	0.09	0.12
	fresh, with pharmaceuticals	1.85	1.91	1.56	1.56	1.46
	after 4-months composting	0.02	0.06	0.38	0.16	0.41
6	fresh	0.00	0.00	0.07	0.04	0.02
	fresh, with pharmaceuticals	2.50	2.09	1.58	1.44	0.74
	after 4-months composting	0.02	0.04	0.15	0.05	0.02
7	fresh	0.00	0.00	0.02	0.01	0.00
	fresh, with pharmaceuticals	1.88	1.38	1.61	1.34	1.67
	after 4-months composting	0.01	0.02	0.02	0.01	0.00

Table 4.4. The extent of degradation of pharmaceuticals in compost mixtures during 4-months composting period

Pile No	Degradation after 4 months of composting, %				
	SMX	SDM	NOR	CIP	OFL
1	83	76	90	92	100
2	100	99	96	95	100
3	100	96	82	94	99
4	100	99	91	98	86
5	99	97	79	90	74
6	99	98	91	97	98
7	99	98	99	100	100

The degradation of FQs and SAs takes place during sewage sludge co-composting with sawdust, peat and straw. Additions of sawdust clearly speed up this process, whereas the mixtures with peat and straw perform lower abilities to decompose pharmaceutical residues. No clear evidence was received concerning

the impact of vermicomposting and oil-shale amendments on the speed of degradation of the studied pharmaceuticals. Further studies were composed with the aim of defining the optimum proportions of bulking agents applicable in sewage sludge composting.

4.3 Degradation of some widely used pharmaceuticals during composting (I)

This section is partly taken from the paper Haiba et al., (2017) with the kind permission of Journal of Agronomy Research.

4.3.1 Diclofenac

The results of the analyses indicated that none of the compost samples was originally free of DCF residues (Table 4.5). DCF concentrations were found in relatively low amounts.

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 (Table 4.5) 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 published data (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 concentration of DCF residue had decreased by 51% in compost mixture (No 1) with sludge-sawdust ratios 1:2 (v:v). In the case of compost mixtures (No 2) with the ratios of 1:3 (v:v) the relevant concentration drop was 42%.

Table 4.5. Concentrations of diclofenac in sewage sludge – sawdust compost samples (mg kg^{-1}, dw)

Compound	Mixture No	Before spiking	1 day	1 week	1 month
DCF	1	0.086±0.004	2.646±0.319	1.307±0.035	0.209±0.010
	2	0.064±0.005	2.381±0.212	1.369±0.044	0.036±0.002

Sewage sludge – sawdust ratio in sample 1 was 1:2 and dry matter content – 35.3%; and in sample 2 accordingly 1:3 and 40.8%

According to the data presented in table 4.6 it is evident that the degradation of DCF was more complete when higher ratio of sawdust was used in preparing compost mixtures.

Table 4.6. Extent of degradation (%) for diclofenac during one month composting

Mixture No	DCF
1	92
2	98

These results show clearly that the degradation of DCF almost fully takes place already during one-month composting period in the case of compost samples with the ratios of 1:3 (v:v). Still, the results indicate that longer periods, especially in the case of compost mixtures with sludge-sawdust ratios 1:2 (v:v), are needed for the more complete removal of pharmaceutical residues from sewage sludge based compost.

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. 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.

According to Martínez-Alcalá et al. (2017) DCF undergoes "partial biodegradation", as its biodegradation rate constant K_{biol} = 1.31 L g_{ss}^{-1} h^{-1} (in the case of 0.1 <K_{biol} < 10 L g_{ss}^{-1} h^{-1} the expected biological transformation rate in a WWTP is between 20 and 90%). The value of K_d obtained for DCF was 0.11 L g_{ss}^{-1}. In WWTPs the elimination of DCF fully takes place through sorption in WWTPs.

The fast removal of DCF during composting, observed by Butkovskyi et al. (2016), is not in agreement with the published results on the aerobic degradability of this pollutant (Joss et al., 2006a: $K_{biol} \leqslant$ 0.1 L g_{ss}^{-1} h^{-1}). Transformation of DCD, which is stable towards aerobic and anaerobic biodegradation, is possibly attributed to the activity of fungal biomass (Butkovskyi et al., 2016). The DFC removal efficiencies in WWTPs are in the range from 0% to 80% (Zhang et al., 2008). The rapidness of the removal of DCF in compost mixture could be explained by the differences in microbial composition of compost in comparison to activated sludge at the WWTPs (Langenhoff et al., 2013).The study carried out by Rodarte-Morales et al. (2012) reveals white rot fungus *Phanerochaete chrysosporium* capability of complete degradation of DCF in an aerobic environment (Butkovskyi et al., 2016). The fungal biomass formed 1.25% or more of the total dry matter of compost at the end of the study. Thus, rapid degradation of DCF during the composting process was presumably elaborated by fungi (Butkovskyi et al., 2016).

4.3.2 Carbamazepine

As it can be seen from table 4.7, none of the compost mixtures was free of CBZ. Its concentrations were from 41 to 62 μg kg^{-1}. This data for CBZ is in reasonable agreement with the results published by Miao et al. in 2005. The results clearly

show that no degradation of CBZ took place during composting experiments (see Tables 4.7 and 4.8).

Table 4.7. Concentrations of carbamazepine (CBZ) in sewage sludge – sawdust compost mixtures (mg kg^{-1}, dw)

Compound	Mixture No	Before spiking	1 day	1 week	1 month
CBZ	1	0.062±0.002	3.106±0.383	2.585±0.053	3.201±0.098
	2	0.046±0.003	2.685±0.260	2.314±0.077	2.318±0.079

Sewage sludge – sawdust ratio in sample 1 was 1:2 and dry matter content – 35.3%; and in sample 2 accordingly 1:3 and 40.8%

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 WWTPs CBZ sometimes exhibits negative removal efficiency (Collado et al., 2014).

Table 4.8. Extent of degradation (%) for carbamazepine

Mixture No	CBZ
1	-11
2	13

CBZ readily adsorbs on sludge particles (Blair et al., 2015; Nielsen and Bandosz, 2016). The solid-water distribution coefficient has also been obtained for CAR in mesophilic (35.4 L kg^{-1}) (Carballa et al., 2008), thermophilic (20.2 L kg^{-1}) (Carballa et al., 2008), and secondary (1.2 L kg^{-1}) (Ternes et al., 2004) sludge.

The concentrations of CBZ and metabolites increase on a dry weight basis between untreated and treated biosolids (Miao et al., 2005). 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., 2013b). 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). 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. (2013b) the values of $t_{1/2}$ for CBZ in soils were between 46 and 173 days. The calculated by Martínez-Alcalá et al. (2017) $K_{biol} = -0.87$ L g_{ss}^{-1} h^{-1} and $K_d = 0.47$ L g_{ss}^{-1}.

4.3.3 Metformin

The concentrations of MET in the mixtures were very low before spiking: 1 to 2 μg kg^{-1} (Table 4.9). The same cannot be said about the other studied pollutants. The results given in tables 4.9 and 4.10 show that more than 90% of MET degrades during a 1-month composting period.

Table 4.9. Concentrations of metformin (MET) in sewage sludge – sawdust mixtures (mg kg^{-1}, dw)

Compound	Mixture No	Before spiking	1 day	1 week	1 month
MET	1	0.002±0.000	2.137±0.250	0.442±0.015	0.181±0.010
	2	0.001±0.000	1.952±0.152	0.299±0.015	0.140±0.016

Sewage sludge – sawdust ratio in sample 1 was 1:2 and dry matter content – 35.3%; and in sample 2 accordingly 1:3 and 40.8%

Table 4.10. Extent of degradation (%) for MET during one month composting

Mixture No	MET
1	91
2	93

According to Mrozik and 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). This compound is polar and very soluble in water; thus it interacts more strongly with water than with the soil surface. Although its half-lives were 1−5 days in different soils, due to its weak sorption MET may be a potential threat to ground and surface water (Benotti and Brownawell, 2005; 2008). For MET the role of biodegradation and sorption can be expressed with the values of K_{biol} and K_d: K_{biol} = 0.54 L g_{ss}^{-1} h^{-1} and K_d = 3 L g_{ss}^{-1} (Blair et al., 2015). Interestingly, MET stopped being degraded at notable levels within an activated sludge wastewater treatment process (Blair et al., 2015).

4.3.4 Triclosan

The initial concentrations of TCS in compost were up to 2 mg kg^{-1} (dw) (Table 4.11). After adding TCS to the compost samples its concentrations were determined again, and they were clearly above the expected values (Table 4.11) 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 concentration of TCS had decreased by 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 drop was only 28% (Table 4.11).

Table 4.11. Concentrations of triclosan (TCS) in sewage sludge – sawdust compost mixtures (mg kg^{-1}, dw)

Compound	Mixture No	Before spiking	1 day	1 week	1 month
TCS	1	**1.768±0.062**	4.541±0.378	3.241±0.202	**2.068±0.138**
	2	**1.232±0.070**	3.528±0.143	2.538±0.089	**0.682±0.019**

Sewage sludge – sawdust ratio in sample 1 was 1:2 and dry matter content – 35.3%; and in sample 2 accordingly 1:3 and 40.8%

According to the data presented in table 4.12 it is evident that the degradation of TCS was more complete when higher ratio of sawdust was used in preparing compost mixtures. Still the level of degradation was clearly insufficient. 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.

Table 4.12. Extent of degradation (%) triclosan during one month composting

Mixture No	TCS
1	55
2	81

Recent studies show that TCS is not fully degraded in WWTPs (Olaniyan et al., 2016; Tohidi and Cai, 2017). The same is valid in the case of composting with waste wood (Butkovskyi et al., 2016). Adsorption to sludge and biodegradation are considered as two main processes for TCS elimination in WWTPs (Tohidi and Cai, 2017). According to Sadef et al. (2014) the optimal TCS conversion seems to take place at temperatures of 30–50 °C. It was suggested that for TCS, different microorganisms were responsible for removal of TCS. The results obtained by Sadef et al. confirmed that removal of organic pollutants during composting does occur but the processes may have different temperature dependencies.

TCS showed a strong affiliation to all the sediments with linear adsorption coefficients (K_d) that varied from 220 to 1092 L g^{-1}, and the adsorption capacity was related to the total organic carbon (TOC) contents of the sediments. The half-lives of TCS varied from 55 to 239 days, and were longer in sediment with higher K_d (Huang et al., 2015). Both the dependence on temperature and variations in adsorption make the degradation process of TCS strongly dependent on the conditions under which the composting of sewage sludge takes place.

The work carried out by Carr et al. (2011) showed that TCS degraded by microbial populations in soils under both aerobic and reduced oxygen conditions. The corresponding half-lives were 5.9 vs. 8.9 days. The half-life reported by Ying and Kookana (2007) was 18 days. According to Ying et al. (2003) no degradation of TCS takes place under anaerobic conditions. In contrast, Carr et al. observed reasonable rates of microbial degradation with half-lives between 15.3 and

28.8 days under anaerobic conditions. Over the 14-day study, between 27% and 40% of the added TCS was lost due to microbial degradation (Carr et al., 2011).

4.4 Composting and microbiological indices (I)

In the beginning of the experiment the growth of microbial population caused the rise of temperature drastically in compost samples with pharmaceuticals (samples 1 and 3), if compared to reference samples (2 and 4) (Fig. 4.1). Although SIR profiles looked similar in the case of all four compost samples (described in detail in Haiba et al., 2017), the highest temperatures in compost samples 1 (57.5 °C) and 3 (52.5 °C) differed from the temperature peaks in samples 2 (42.2 °C) and 4 (41.4 °C), more than 10 ºC. The reason for that might have been the difference in the ratios between fungi and bacteria (table 3 in Haiba et al., 2017). Compost samples with pharmaceuticals (samples 1 and 3) had a lower ratio of fungi and bacteria (0.974 and 0.909) compared to the reference compost samples (sample 2 – 0.980 and sample 4 – 0.965). 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 essential that in the beginning of the experiment the temperature rose faster in compost samples 1 and 3 (Haiba et al., 2017).

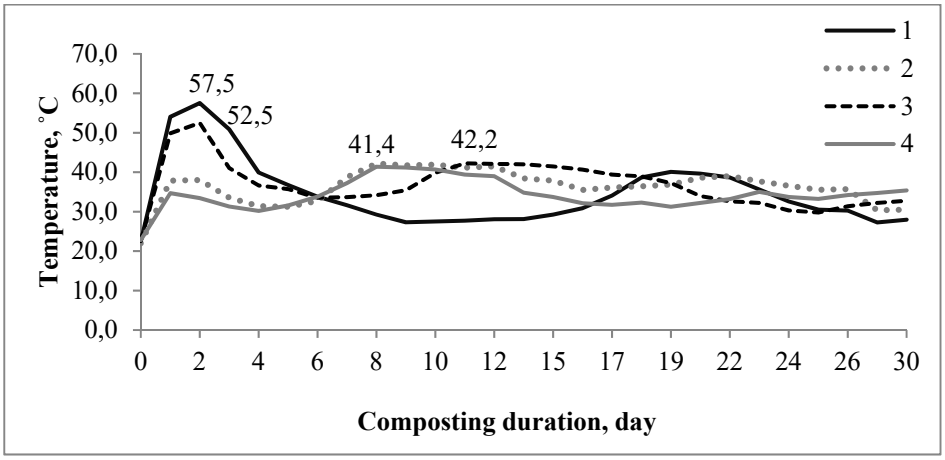

Figure 4.1. Temperature profiles during one month composting for 1:2 (v:v) sewage sludge – sawdust mixtures: 1 – containing PPCPs; 2 – without PPCPs and for 1:3 (v:v) sewage sludge – sawdust mixtures: 3 – containing PPCPs; 4 – without PPCPs.

After one week the ratio of fungi and bacteria was reduced in compost samples with added pharmaceuticals, but biomass of microorganisms had increased in the case of samples 2 and 4. It could be the reason for higher temperatures in samples 2 and 4 (Fig. 4.1) (Haiba et al., 2017).

The results of the experiment showed that the PPCP residues in the compost mixtures did not have an acute toxic effect on microorganisms. The PPCPs with the solution added to the compost mixtures (samples 1 and 3) made the composting processes more unstable, which made the microbiological parameters fluctuate at much higher amplitude than the reference composts (samples 2 and 4). The added contaminants were not permanently harmful to microorganisms, causing only temporary stress, and therefore the rates of microbial biomass and respiration activity began to increase rapidly at the end of the first week. However, constant exposure with various compounds and their residues in small doses may result in resistance of microorganisms (Clarke and Smith, 2011; Kinney et al., 2008; Lee et al., 2012). Microorganisms are at the base of the soil food web and effects on microbial communities translate to higher trophic levels represented by soil fauna organisms (Coors et al., 2016). A large part of the antibiotic resistance genes adsorbes during sewage treatment processes on sewage sludge particles (Bondarczuk et al., 2016) and then to soil (Kim and Aga, 2007). It is important to reduce the amount of contaminating resistance genes in sewage sludge and its compost before reuse (Chen et al., 2016).

4.5 On the degradation of selected pharmaceuticals

For degradation experiments, data 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).

The degradation of MET takes place rapidly and fully both in soils (from Mrozik and 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. Further work on toxicity assessments for guanylurea is needed (Trautwein and Kümmerer, 2011). In the case of different soils there is a 99 … 100% degradation of MET during a 30-day perios, whereas in the studied compost mixture degradation is lower at 92 … 93%.

Previous research has shown that 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). The bioconcentration factors found for DCF are 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 = 0.010 d^{-1} and $t_{1/2}$ = 70 d, and for compost mixtures (current study) k = 0.09 … 0.1 d^{-1} and $t_{1/2}$ = 7 … 8 d. In sterile soil only 26% of DCF degrades during a 30-day period, whereas in compost mixtures the level of degradation was 92 … 98%.

TCS gives the following k and $t_{1/2}$ values in the case of agricultural soils (Xu et al., 2009): 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). 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).

The degradation studies clearly show that the persistence of pharmaceuticals in the studied compost mixtures increases in the row MET→DFC→TCS→CBZ. Interestingly, the degradation of TCS in sterile soil is faster than the degradation of DCF. In compost mixtures DCF degrades almost fully during a 30-day composting period. In compost mixtures, as compared to sterile soil, the rate of degradation of DCF is much higher durng the 30-day period compared to the rate of degradation of TCS. This suggests that the main route of the TCS degradation does not go through microbial processes. Slight increase in the degradation rate of TCS in compost mixtures, if compared to the degradation in sterile soil, can be explained by elevated temperatures that occur during the formation of compost. The results obtained in the case of agricultural soils support this conclusion (Xu et al., 2009).

CBZ was an exception among the studied pharmaceuticals: this compound was persistent under all studied conditions. This leads to the conclusion that composting is not an appropriate mean for degrading this compound.

Analysis of results on the degradation of pharmaceuticals available in the relevant papers or obtained as a result of the current study led to some general considerations:

- 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 takes time.
- The optimization of composting technologies resulted in the efficient degradation of DCF, MET and TCS, while for the elimination of CBZ from sewage sludge different measures should be undertaken.
- In sterile soils the degradation of pharmaceuticals is commonly slow, leading to the conclusion that the main pathway of their degradation goes through microbial processes.
- 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.
- Although the plant uptake of highly soluble compounds (as MET and SAs) readily takes place, their concentrations in soil or sewage sludge compost are low, and consequently, they commonly do not generate severe problems associated with food toxicity.
- In many cases, the degradation of pharmaceuticals in soil and compost follows the first-order exponential decay model. Still, not all of the widely

used medical compounds (as for example CBZ) follow this rule. Some of them are relatively persistent.

- No comprehensive approaches exist for calculating the reliable concentrations of pharmaceuticals that have been a subject of biodegradation, but the ongoing work is bringing closer the creation of more appropriate models.

CONCLUSIONS

This study was to find reliable ways of optimizing sewage sludge treatment technologies aimed to the need of increasing the environmental safety of the resulting compost. The main focus of this thesis is on the treatment of sewage sludge via composting and the degradation of pharmaceuticals during this process. Means of reducing the impact of some widely used pharmaceuticals on the environment and people are proposed.

The main results of the study can be summarized as follows:

- In the case of several pharmaceuticals it is possible to enhance their degradation rate through the intelligent selection of sewage sludge composting conditions.
- The degradation of FQs and SAs takes place during sewage sludge co-composting with sawdust, peat and straw. Additions of sawdust clearly sped up this process, whereas the mixtures with peat and straw perform lower abilities to decompose pharmaceutical residues.
- The degradation of DCF is almost complete during one-month composting period in the case of compost samples with the ratios of 1:3 (v:v). Longer periods, especially in the case of compost mixtures with sludge-sawdust ratios 1:2 (v:v), are needed for the more complete removal of pharmaceutical residues from sewage sludge based compost.
- No degradation of CBZ takes place during composting experiments.
- 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.
- The degradation of TCS was more complete when higher ratio of sawdust was used in compost mixtures. During a 1-month composting period 55% of TCS was degraded in the "1:2" mixture and 81% in the "1:3" mixture. For TCS the half-life had double difference depending on the ratio of sewage sludge and bulking agent (sawdust). Half-life was 13 days for the compost mixture with ratio of 1:3 (v:v) and 26 days for the compost mixture with ratio of 1:2 (v:v).
- The results of this study show that the optimization of composting technologies allows for the efficient degradation of DCF, MET and TCS, whereas for the elimination of CBZ from sewage sludge different means should be used. The composting period should last at least six months.

Overall, the results obtained in this study provide information for choosing intelligent approaches to sewage sludge composting with the aim of degrading pharmaceutical residues present in this media. Although sewage sludge composting is an efficient way for degrading several widely used pharmaceuticals, some of these compounds are highly persistent towards bioprocesses and their elimination needs the application of different measures.

REFERENCES

Al-Rajab, A.J., Sabourin, L., Lapen, D.R., Topp, E. (2010). The non-steroidal anti-inflammatory drug diclofenac is readily biodegradable in agricultural soils. Science of the Total Environment, 409, pp 78–82.

Ammari, T.G., Al-Omari, Q., Abbassi, B.E. (2012). Composting sewage sludge amended with different sawdust proportions and textures and organic waste of food industry – assessment of quality. Environmental Technology, 33, pp 1641–1649.

Amouzgar, P., Salamatinia, B. (2015). A Short Review on Presence of Pharmaceuticals in Water Bodies and the Potential of Chitosan and Chitosan Derivatives for Elimination of Pharmaceuticals. Journal of Molecular and Genetic Medicine, S4:001. doi: 10.4172/1747-0862.S4-001

Astover, A. and Rossner, H. (2013). Knowledge report: Phosphorus status of agricultural soils in Estonia. Baltic Forum for Innovative Technologies for Sustainable Manure Management, p 8.

Avisar, D., Primor, O., Gozlan, I., Mamane, H. (2010). Sorption of sulfonamides and tetracyclines to montmorillonite clay. Water, Air & Soil Pollution, 209, pp 439–450.

Aydin, S., Ince, B., Ince, O. (2015). Development of antibiotic resistance genes in microbial communities during long-term operation of anaerobic reactors in the treatment of pharmaceutical wastewater. Water Research, 83, pp 337–344.

Baharoglu, Z. and Mazel, D. (2011). Vibrio cholerae triggers SOS and mutagenesis in response to a wide range of antibiotics: a route towards multiresistance. Antimicrobial Agents and Chemotheraphy, 55, pp 2438–2441.

Banegas, V., Moreno, J.L., Moreno, J.I., García, C., León, G, Hernández, T.(2007). Composting anaerobic and aerobic sewage sludges using two proportions of saadust. Waste Management, 27, pp 1317–1327.

Baquero, F., Martinez, J.L., Canton, R. (2008). Antibiotics and antibiotic resistance in water environments. Current Opinion in Biotechnology, 19, pp 260–265.

Baran, W., Adamek, E., Ziemiańska, J. Sobczak, A. (2011). Effects of the presence of sulfonamides in the environment and their influence on human healt. Journal of Hazardous Materials, 196, pp 1–15.

Barbosa, M.O., Moreira, N.F.F., Riberio, A.R., Pereira, M.F.R., Silva, A.M.T. (2016). Occurrence and removal of organic micropollutants: an overview of the watch list of EU Decision 2015/495. Water Research, 94, pp 257–279.

Bartha, B., Huber, C., Schröder, P. (2014). Uptake and metabolism of diclofenac in Typha latifolia – How plants cope with human pharmaceutical pollution. Plant Science, 227, pp 12–20.

Batt, A.L., Kim, S., Aga, D.S. (2007). Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations. Chemosphere, 68 (3), pp 428–435.

Beausse J. (2004). Selected drugs in solid matrices: A review of environmental determination, occurrence and properties of principal substances. Trends in Analytical Chemistry, 23, pp 753–761.

Benotti, M.J. and Brownawell, B.J. (2005). Detection of pharmaceuticals in an urban estuary and their behavior as wastewater tracers during wet and dry conditions. EPA Final Report. EPA, New York.

Benotti, M.J.and Brownawell, B.J. (2008). Detection and migration of pharmaceuticals in the groundwater of Suffolk County. EPA Final Report. EPA, New York

Bergersen, O., Hanssen, K.Ø., Vasskog, T. (2012). Anaerobic treatment of sewage sludge containing selective serotonin reuptake inhibitors. Bioresource Technology, 117, pp 325–33.

Blair, B., Nikolaus, A., Hedman, C., Klaper, R., Grundl, T. (2015). Evaluating the degradation, sorption, and negative mass balances of pharmaceuticals and personal care products during wastewater treatment. Chemosphere, 134, pp 395-401.

Bock, M., Lyndall,J., Barber,T., Fuchsman,P., Perruchon,E.,Capdevielle, M. (2010). Probabilistic Application of a Fugacity Model to Predict Triclosan Fate during Wastewater Treatment. Integrated Environmental Assessment and Management, 6, pp 393–404.

Bondarczuk, K., Markowicz, A., Piotrowska-Seget, Z. (2016). The urgent need for risk assessment on the antibiotic resistance spread via sewage sludge land application. Environment International. 87, pp 49–55.

Borgman, O. and Chefetz, B. (2013). Combined effects of biosolids application and irrigation with reclaimed wastewater on transport of pharmaceutical compounds in arable soils. Water Research, 47, pp 3431–3443.

Boxall, A.B.A., Johnson, P., Smith, E.J., Sinclair, C.J., Stutt, E., Levi, L.S. (2006). Uptake of veterinary medicines from soil into plants. Journal of Agricultural and Food Chemistry, 54, pp 2288–2297.

Bridle, T. and Skrypski-Mantele, S. (2000). Assessment of sludge reuse options: a life cycle approach. Water Science and Technology, 4, pp 131–135.

Butkovskyi, A., Ni, G., Hernandez Leal, L., Rijnaarts, H.H.M., Zeeman, G. (2016). Mitigation of micropollutants for black water application in agriculture via composting of anaerobic sludge. Journal of Hazardous Materials, 303, pp 41–47.

Camacho-Munoz D., Martin J., Santos J.L., Aparicio I., Alonso E., (2010). Occurrence, temporal evolution and risk assessment of pharmaceutically active compounds in Doñana Park (Spain), Journal of Hazardous Materials, 183, pp 602–608.

Carballa, M., Fink, G., Omil, F., Lema, J.M., Ternes, T., (2008). Determination of the solid–water distribution coefficient (Kd) for pharmaceuticals, estrogens and musk fragrances in digested sludge. Water Research, 42, pp 287–295.

Carballa, M., Omil, F., Lema, J.M., Llompart, M., García-Jares, C., Rodríguez, I., Gómez, M, Ternes, T. (2004). Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Research, 38, pp 2918–2926.

Carmosini, N. and Lee, L.S. 2008. Sorption and degradation of selected pharmaceuticals in soil and manure. In: Fate of Pharmaceuticals in the Environment and in Water Treatment Systems. Aga, D.S. (ed). CRC Press, Boca Raton, FL, USA, pp. 139–165.

Carr, D.L., Morse, A.N., Zak, J.Z., Anderson, T.A. (2011). Microbially Mediated Degradation of Common Pharmaceuticals and Personal Care Products in Soil Under Aerobic and Reduced Oxygen Conditions. Water, Air, & Soil Pollution, 216, pp 633–642.

Carter, L.J., Harris, E., Williams, M., Ryan, J.J., Kookana, R.S., Boxall, A.B.A. (2014). Fate and uptake of pharmaceuticals in soil-plant systems. *Journal of Agricultural and Food Chemistry*, 62 (4) pp. 816–825.

Casado, J., Castro, G., Rodríguez, I., Ramil, M., Cela, R. (2015). Selective extraction of antimycotic drugs from sludge samples using matrix solid-phase dispersion followed by on-line clean-up. Analytical and Bioanalytical Chemistry, 407, pp 907–917.

Castiglioni, S., Fanelli, R., Calamari, D., Bagnati, R., Zuccato, E. (2004). Methodological approaches for studying pharmaceuticals in the environment by comparing predicted and measured concentrations in River Po, Italy. Regulatory Toxicology and Pharmacology, 39(1) pp 25–32.

Chefetz, B., Mualem, T., Ben-Ari, J., (2008). Sorption and mobility of pharmaceutical compounds in soil irrigated with reclaimed wastewater. Chemosphere, 73, pp 1335–1343

Chen, G., den Braver, M.W., van Gestel C.A.M, van Straalen N.M., Roelofs, D. (2015). Ecotoxicogenomic assessment of diclofenac toxicity in soil. Environmental Pollution, 199, pp 253–260.

Chen, L., De Haro, M.M., Moore, A., Falen, C. (2011a). The Composting Process: Dairy Compost Production and Use in Idaho CIS 1179. University of Idaho, 5 pages.

Chen,X., Nielsen, J.L., Furgal, K., Liu , Y., Lolas, I.H., Bester, K. (2011b). Biodegradation of triclosan and formation of methyl-triclosan in activated sludge under aerobic conditions. Chemosphere. 84, pp 452–456.

Chen, Y., Yu, G., Cao, Q., Zhang, H., Lin, Q. & Hong, Y. (2013). Occurrence and environmental implications of pharmaceuticals in Chinese municipal sewage sludge. Chemosphere, 93, pp 1765–72.

Chen, Y., Yu, F., Liang, S., Wang, Z., Liu, Z., Xiong, Y. (2014). Utilization of solar energy in sewage sludge composting: Fertilizer effect and application. Waste Management 34, pp 2014–2021.

Chen, Q., An, X., Li, H., Su, J., Ma, Y., Zhu, Y-G. (2016). Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. Environment International. 92, pp 1–10.

Christou, A., Karaolia, P., Hapeshi, E., Michael, C., Fatta-Kassinos, D. (2017). Long-term wastewater irrigation of vegetables in real agricultural systems: Concentration of pharmaceuticals in soil, uptake and bioaccumulation in tomato fruits and human health risk assessment. *Water Research*, 109, pp 24–34.

Chroni, C., Kyriacou, A., Manios, T., Lasaridi, K-E. (2009). Investigation of the microbial community structure and activity as indicators of compost stability and composting process evolution. Bioresource Technology 100, pp 3745–3750.

Clarke, B.O., Smith, S.R. (2011). Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. Environment International.37, pp.226–247.

Collado, N., Rodriguez-Mozaz, S., Gros, M., Rubirola, A., Barcelo, D., Comas, J., Rodriguez-Roda, I., Buttiglieri, G., (2014). Pharmaceuticals occurrence in a WWTP with significant industrial contribution and its input into the river system. Environmental Pollution, 185, pp 202−212.

Colon, B. and Toor, G.S. (2016). A Review of Uptake and Translocation of Pharmaceuticals and Personal Care Products by Food Crops Irrigated with Treated Wastewater. In: Donald L Sparks, editors: Advances in Agronomy, Vol 140, Chennai: Academic Press; 2016, pp. 75–100. ISBN:978-0-12-804691-3 © Copyright 2016 Elsevier Inc. Academic Press.

Coors, A., Edwards, M., Lorenz , P., Römbke, J., Schmelz, R.M., Topp , E., Waszak, K., Wilkes, G., Lapen, D.R. (2016). Biosolids applied to agricultural land: Influence on structural and functional endpoints of soil fauna on a short- and long-term scale. Science of the Total Environment.562, pp 312–326.

Cukjati, N., Zupancic, G.D., Ros, M., Grilc, V. (2012). Composting of anaerobic sludge: An economically feasible element of a sustainable sewage sludge management. Journal of Environmental Management, 106, 48–55.

Cunningham, V.L., D'Aco, V.J., Pfeiffer, D., Anderson, P.D., Buzby, M.E., Hannah, R.E., Jahnke, J., Neil J Parke, N.J. (2011). Predicting Concentrations of Trace Organic Compounds in Municipal Wastewater Treatment Plant Sludge and Biosolids Using the PhATETM Model. Integrated Environmental Assessment and Management, 8, pp 530–542.

Dalkmann, P., Broszat, M., Siebe, C., Willaschek, E., Sakinc, T., Huebner, J., Amelung, W., Grohmann, E., Siemens, J. (2012). Accumulation of pharmaceuticals, *Enterococcus*, and resistance genes in soils irrigated with wastewater for zero to 100 years in Central Mexico. *PLoS ONE* 7 (9), e45397.

Daughton, C.G. and Ternes, T.A. (1999). Pharmaceuticals and personal care products in the environment: agents of subtle change? Environmental Health Perspectives, 107, pp 907–938.

Davies, J. (1994). Inactivation of antibiotics and the dissemination of resistance genes. Science, 264, pp 375–382.

De Lucia, B. Cristiano, G. Vecchietti, L., Bruno, L. (2013). Effect of different rates of composted organic amendment on urban soil properties, growth and nutrient status of three Mediterranean native hedge species. Urban Forestry & Urban Greening, 12, pp 537–545.

Decree of Estonian Minister of the Environment No.78. 2004. State Gazette I, 9, 52, 2004, Estonia (in Estonian)

Dobor, J., Varga M., Yao J., Chen H., Palkó G., Záray G. (2010). A new sample preparation method for determination of acidic drugs in sewage sludge applying microwave assisted solvent extraction followed by gas chromatography–mass spectrometry. Microchemical Journal, 94, pp 36–41.

Dolliver H, Kumar K, Gupta SC. (2007). Sulfamethazine uptake by plants from manure-amended soil. Journal of Environmental Quality, 36, pp 1224–1230.

Donner, E., Kosjek, T., Qualmann, S., Kusk, K.O., Heath, E., Revitt, D.M., Ledin, A., Andersen H.R. (2013). Ecotoxicity of carbamazepine and its UV photolysis transformation products. Science of The Total Environment, 443, pp 870–876.

Doorslaer, X.V., Dewulf, J., Van Langenhove, H., Demeestere, K. (2014). Fluoroquinolone antibiotics: An emerging class of environmental micropollutants. Review article. Science of the Total Environment, 500–501, pp 250–269.

Drugbank. Sulfadimethoxine. https://www.drugbank.ca/drugs/DB06150

Durán-Álvareza, J.C., Pradob, B., Gonzálezc, D., Sánchezc, Y., Jiménez-Cisnerosd, B. (2015). Environmental fate of naproxen, carbamazepine and triclosan in wastewater, surface water and wastewater irrigated soil — results of laboratory scale experiments. Science of the Total Environment, 538, pp 350–362.

Eggen, T., Asp T.N., Grave, K., Hormazabal, V. (2011). Uptake and translocation of metformin, ciprofloxacin and narasin in for age and crop plants. Chemosphere, 85, pp 26–33.

EMEA/CVMP/055/96. 1996. Committee for Veterinary Medicinal Products. Note for guidance: environmental risk assessment for veterinary medicinal products other than GMO-containing and immunological products. Veterinary Medicines Evaluation Unit, London, 1996.

EU Council Directive 86/278/EEC. 1986. On the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. European Community, Brussels, 12 June 1986.

Fu, Q., Wu, X., Ye, Q., Ernst, F., Gan, J. (2016). Biosolids inhibit bioavailability and plant uptake of triclosan and triclocarban. *Water Research* 102, pp 117–124.

Garcia-Rodriguez, A., Sagrista, E., Matamoros, V., Fontas, C., Hidalgo, M., Salvado, V. (2014). Determination of pharmaceutical compounds in sewage sludge using a standard addition method approach. International Journal of Environmental Analytical Chemistry, 94, pp 1199–1209.

Gielen, G.J.H.P. (2007). PhD thesis.The fate and effects of sewage-derived pharmaceuticals in soil. University of Canterbury, P 189.

Girardi, C., Greveb, J., Lamshöftc, M., Fetzerd, I., Miltnera, A., Schäffere, A., Kästner, M. (2011). Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities. Journal of Hazardous Materials, 198, pp 22–30.

Göbel, A., Thomsen, A., McArdell, C.S., Joss, A., Giger, W. (2005). Occurrence and Sorption Behavior of Sulfonamides, Macrolides, and Trimethoprim in Activated Sludge Treatment. Environmental Science and Technology, 39, 3981-3989.

Goldstein, M.; Shenker, M.; Chefetz, B. (2014).Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables. Environmental Science & Technology, 48, pp 5593−5600.

Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C., Giger, W. (2003). Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. Environmental Science and Technology, 37, pp 3243–3249.

Golovko, O., Kumar, V., Fedorova, G., Randak, T., Grabic, R., (2014). Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere, 111, pp 418–426.

Grossberger, A., Hadar, Y., Borch, T., Chefetz, B. (2014). Biodegradability of pharmaceutical compounds in agricultural soils irrigated with treated wastewater. *Environmental Pollution*, 185, pp 168−177.

Haiba, E., Lillenberg, M., Kipper, K., Astover, A., Herodes, K., Ivask, M., Kuu, A., Litvin, S.V., Nei, L. (2013a). Fluoroquinolones and sulfonamides in sewage sludge compost and their uptake from soil into food plants. African Journal of Agricultural Research, 8, pp 3000–3006

Haiba, E., Nei, L., Lillenberg, M., Kipper, K. & Herodes, K. (2013b). Degradation of some pharmaceuticals during sewage sludge composting. Global Journal on Advances Pure and Applied Sciences, 1st Global Conference on Environmental Studies (CENVISU-2013), Belek, Antalya, Turkey, 1, pp 857−862.

Haiba, E., Ivask, M., Olle, L., Peda, J., Kuu, A., Kutti, S., Nei, L. (2014). Transformation of nutrients and organic matter in vermicomposting of sewage sludge and kitchen wastes. Journal of Agricultural Science, 6, pp 114–118.

Haiba, E., Nei, L., Ivask, M., Peda, J., Järvis, J., Lillenberg, M., Kipper, K., Herodes, K. (2016). Sewage sludge composting and fate of pharmaceutical residues – recent studies in Estonia. Agronomy Research, 14, pp 1583−1600.

Haiba, E., Nei, L., Kutti, S., Lillenberg, M., Herodes, K., Ivask, M., Kipper, K., Aro, R., Laaniste, A. (2017). Degradation of diclofenac and triclosan residues in sewage sludge compost. Agronomy Research, 15, pp 395−405.

Haiba, E. and Nei, L. (2017). Sewage sludge composting and pharmaceuticals. European Scientific Journal, pp 114−121.

Hamscher, G., Sczesny, S., Höper, H., Nau, H. (2002). Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Analytical Chemistry, 74, pp 1509−1518.

Hara, K. and Mino, T. (2008). Environmental assessment of sewage sludge recycling options and treatment processes in Tokyo. Waste Management, 28, pp 2645–2652.

Harrison, E.Z., Oakes, S.O., Hysell, M., Hay, A. (2006). Organic chemicals in sewage sludges. Review. Science of the Total Environment, 367, pp 481–497.

Hawker, D.W. and Connell, D.W. (1988). Octanol-water partition coefficients of polychlorinated biphenyl congeners. Environmental Science & Technology, 22, pp 382–387.

Ho, Y. B., Zakaria, M. P., Latif, P. A., Saari, N. (2013). Degradation of veterinary antibiotics and hormone during broiler manure composting. Bioresource Technology, 131, pp 476–484.

Holling, C.S., Bailey, J.L., Heuvel, B.V., Kinney, C.A. (2012). Uptake of human pharmaceuticals and personal care products by cabbage (Brassica campestris) from fortified and biosolids-amended soils. Journal of Environmental Monitoring, 14, pp 3029−3036.

Hooper, D.C. and Wolfson, J.S. 1991. Fluoroquinolone antimicrobial agents. The New England Journal of Medicine, 324, pp 384–394.

Huang, X., Wu, C., Hu, H., Yu, Y., Liu, J. (2015). Sorption and degradation of triclosan in sediments and its effect on microbes. Ecotoxicology and Environmental Safety, 116, pp 76–83.

Huber, C., Bartha, B., Schröder, P. (2012). Metabolism of diclofenac in plants – Hydroxylation is followed by glucose conjugation. Journal of Hazardous Materials, 243, pp 250– 256.

Ingerslev, F. and Halling-Sørensen, B. (2000). Biodegradability properties of sulfonamides in activated sludge. Environmental Toxicology and Chemistry, 19, pp 2467–2473.

Iqbal, M. K., Nadeem, A., Sherazi, F., Khan, R.A. (2015). Optimization of process parameters for kitchen waste composting by response surface methodology. International Journal of Environmental Science and Technology, 12, pp 1759–1768

Järvis, J., Ivask, M., Nei, L., Kuu, A., Luud, A. (2016). Effect of Green Waste Compost Application on Afforestation Success. Baltic Forestry, 22, pp 90–97.

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sa´nchez, R., Ventura, F., Petrovic, M., Barcelo, D. (2011). Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Research, 45, pp 1165–1176.

Jelić, A., Petrović, M., Barceló D. (2009). Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry. Talanta, 80, pp 363–371.

Jjemba, P.K. (2002). The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land: a review. Agriculture, Ecosystems & Environment, 93, pp 267−278.

Joss, A., Zabaczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H. (2006a). Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. Water Research, 40, pp. 1686-1696.

Joss, A., Carballa, M., Kreuzinger, N., Siegrist, H., Zabczynski, S., (2006b). Wastewater treatment. In: Ternes, T.A., Joss, A. (Eds.), Human Pharmaceuticals, Hormones and Fragrances: the Challenge of Micropollutants in UrbanWater Management. IWA Publishing, London, UK.

Khan, S.J. and Ongerth, J.E. (2004).Modelling of pharmaceutical residues in Australian sewage by quantities of use and fugacity calculations. Chemosphere, 54(3) pp 355–367.

Kim, K.-R., Owens, G., Ok, Y.S., Park, W.-K., Lee, D.B., Kwon, S.-I. (2012). Decline in extractable antibiotics in manure-based composts during composting. Waste Management, 32, pp. 110–116.

Kim, S. and Aga, D.S. (2007). Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants. Journal of Toxicology and Environmental Health. 10, pp 559–573.

Kinney, C., Furlong, E., Kolpin, D., Burkhardt, M., Zaugg, S. (2008). Bioaccumulation of Pharmaceuticals and Other Anthropogenic Waste Indicators in Earthworms from Agricultural Soil Amended With Biosolid or Swine Manure. Environmental Science & Technology.42 pp, 1863–1870.

Kipper, K., Herodes, K., Lillenberg, M., Nei, L., Haiba, E., Litvin, S.V. (2010). Plant Uptake of some Pharmaceuticals Commonly Present in Sewage Sludge Compost. Conference: 2nd International conference on Chemical, Biological and Environmental Engineering, pp 261–264.

Kipper, K., Herodes, K., Leito, I., Nei, L. (2011). Two fluoroalcohols as components of basic buffers for liquid chromatography electrospray ionization mass spectrometric determination of antibiotic residues. Analyst, 136, pp 4587−4594.

Kipper, K. (2012) Fluoroalcohols as components of LC-ESI-MS eluents: usage and applications. PhD thesis. University of Tartu.

Kipper, K., Lillenberg, M., Herodes, K., Nei, L., Haiba, E. (2017). Simultaneous Determination of Fluoroquinolones and Sulfonamides Originating from Sewage Sludge Compost. The Scientific World Journal, volume 2017, 8 pages.

Knapp, C.W., Dolfing, J., Ehlert, P.A.I., Graham, D.W. (2010). Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environmental Science & Technology, 44, pp 580–587.

Knepper, T.P. and Barceló, D. (2003). Chapter 8 Recommendations and future Trends. Review article. Comprehensive Analytical Chemistry, 40, pp 927-936.

Koba, O., Golovko, O., Kodesova, R., Klement, A., Grabic, R. (2016). Transformation of atenolol, metoprolol, and carbamazepine in soils: The identification, quantification, and stability of the transformation products and further implications for the environment. Environmental Pollution, 218 pp 574–585.

Komilis, D., Evangelou, A., Voudrias, E. (2011). Monitoring and optimizing the co-composting of dewatered sludge: A mixture experimental design approach. Journal of Environmental Management, 92, pp 2241–2249.

Langenhoff, A., Inderfurth, N., Veuskens, T., Schraa, G., Blokland, M., Kujawa-Roeleveld, K., Rijnaarts, H. (2013). Microbial removal of the pharmaceutical compounds ibuprofen and diclofenac from wastewater. BioMed Research International, 2013, Article ID 325806, 9 pages [WWW] http://dx.doi.org/10.1155/2013/325806

Lazcano, C., Gómez-Brandón, M.,. Domínguez, J. (2008). Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. Chemosphere, 72, pp 1013–1019

Leclercq, M., Mathieu, O., Gomez, E., Casellas, C., Fenet, H., Hillaire-Buys, D. (2009). Presence and fate of carbamazepine, oxcarbazepine, and seven of their metabolites at wastewater treatment plants. Archives of Environmental Contamination and Toxicology 56, pp 408−415.

Lee, D.G., Zhao, F., Rezenom, J.H., Russell, D.H., Chu, K-H. (2012). Biodegradation of triclosan by a wastewater microorganism. Water research.46, pp 4226–4234.

Li, B. and Zhang, T. (2010). Biodegradation and adsorption of antibiotics in the activated sludge process. Environmental Science & Technology, 44, pp 3468–3473.

Li, J.Y., Dodgen, L., Ye, Q.F., Gan, J., (2013b). Degradation kinetics and metabolites of carbamazepine in soil. Environmental Science & Technology, 47, pp 3678−3684.

Li, Y., Li, W., Liu, B., Wang, K., Su, C. Wu, C. (2013a). Ammonia emissions and biodegradation of organic carbon during sewage sludge composting with different ekstra carbon sources. International Biodeterioration & Biodegradation, 85, pp 624–630.

Li, Y., Zhu, G., Jern, Ng.W., Tan, S.K. (2014). A review on removing pharmaceutical contaminants from waste water by constructed wetlands: Design, performance and mechanism. Science of the Total Environment, 468–469, pp 908–932.

Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Sepp, K., Lõhmus, R., Nei, L. (2009). Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry. Journal of Chromatography A, 1216, pp 5949–5954.

Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Lõhmus, R., Ivask, M., Kuu, A., Kutti, S., Litvin, S.V., Nei, L. (2010a). Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting. International Journal of Environmental Science & Technology, 7, pp 307–312.

Lillenberg, M., Litvin, S. V., Nei, L., Roasto, M., Sepp, K. (2010b). Enrofloxacin and Ciprofloxacin Uptake by Plants from Soil. Agronomy Research, 8, pp 807–814.

Lillenberg, M. (2011). Residues of some pharmaceuticals in sewage sludge in Estonia, their stability in the environment and accumulation into food plants via fertilizing: PhD thesis in environmental protection. Estonian University of Life Sciences. Tartu: Ecoprint.

Loos, R., Carvalho, R., António, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D., Gawlik, B.M. (2013). EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Research, 47, pp 6475–6487.

Lozano,N., Rice, C.P., Ramirez, M., Torrents, A. (2010). Fate of triclosan in agricultural soils after biosolid applications. Chemosphere, 78, pp 760–766.

Luo, Y., Guoa, W., Ngo, H.H., Nghiem, L.D., Hai, F.I, Zhang, J., Liang, S., Wang, X.C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Science of the Total Environment, 473–474, pp 619–641.

Mäesaar, M., Kramarenko, T., Meremäe, K., Sõgel, J., Lillenberg, M., Häkkinen, L., Ivanova, M., Kovalenko, K., Hõrman, A., Hänninen, M-L., Roasto, M. (2016). Antimicrobial resistance profiles of Campylobacter spp. isolated from broiler chicken meat of Estonian, Latvian and Lithuanian origin at Estonian retail level and from patients with severe enteric infections in Estonia. Zoonoses and Public Health, 63, pp 89−96.

Malarvizhi, A., Kavitha, C., Saravanan, M., Ramesh, M. (2012). Carbamazepine (CBZ) induced enzymatic stress in gill, liver and muscle of a common carp, Cyprinus carpio. Journal of King Saud University – Science, 24, pp 179–186.

Malchi, T., Maor, Y., Tadmor, G., Shenker, M., Chefetz, B. (2014).Irrigation of root vegetables with treated wastewater: evaluating uptake of pharmaceuticals and the associated human health risks. Environmental Science & Technology, 48, pp 9325−9333

Malinska, K., Zabochnicka-Świątek, M., Dach, J. (2014). Effects of biochar amendment on ammonia emission during composting of sewage sludge. Ecological Engineering, 71, pp 474–478.

Margesin, R., Cimadom, J., Schinner, F. (2006). Biological activity during composting of sewage sludge at low temperatures. International Biodeterioration & Biodegradation, 57(2), pp 88–92.

Markiewicz, M., Jungnickel, C., Stolte, S., Białk-Bielińska, A., Kumirska, J., Mrozik, W. (2017). Primary degradation of antidiabetic drugs. *Journal of Hazardous Materials*, 324, pp 428–435.

Martín, J., Camacho-Munoz, M. D., Santos, J.L., Aparicio, I., Alonso, E. (2012a). Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal. Journal of Hazardous Materials, 239-240, pp 40–47.

Martín, J., Santos, J.L., Aparicio, I., Alonso, E. (2015). Pharmaceutically active compounds in sludge stabilization treatments: anaerobic and aerobic digestion, wastewater stabilization ponds and composting. Science of the Total Environment, 503, pp 97–104.

Martínez-Alcalá, I., Guillén-Navarro, J.M., Fernández-López, C. (2017). Pharmaceutical biological degradation, sorption and mass balance determination in a conventional activated-sludge wastewater treatment plant from Murcia, Spain. Chemical Engineering Journal, 316 pp 332–340.

Maruthur, N.M., Tseng, E., Hutfless, S., Wilson, L.M., Suarez-Cuervo, C., Berger, Z., Chu, Y., Iyoha, E., Segal, J.B., Bolen, S. (2016). Diabetes Medications as Monotherapy or Metformin-Based Combination Therapy for Type 2 Diabetes: A Systematic Review and Meta-analysis. Annals of Internal Medicine, 164, pp 740–751.

Maulini-Duran, C., Artola, A., Font, X., Sánchez, A. (2013). A systematic study of the gaseous emissions from biosolids composting: Raw sludge versus anaerobically digested sludge. Bioresource Technology, 147, pp 43–51.

McClellan, K. and Halden, R.U. (2010). Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey. Water Research, 44, pp 658–668.

Merck Manuals1. Online Medical Library.
http://www.merckmanuals.com/professional/sec14/ch170/ch170f.html

Merck Manuals2. Online Medical Library.
http://www.merckmanuals.com/professional/sec14/ch170/ch170n.html

Merck Manuals3. Online Medical Library.
http://www.merckmanuals.com/professional/sec14/ch170/ch170n.html#sec14-ch170-ch170n-404f

Miao, X-S., Yang, J.-J., Metcalfe, C.D. (2005). Carbamazepine and its metabolites in wastewater and in biosolids in a municipal wastewater treatment plant. Environmental Science & Technology, 2005, pp 7469–7475.

Michelini, L., Reichel, R., Werner, W., Ghisi, R., Thiele-Bruhn, S. (2012). Sulfadiazine uptake and effects on Salix fragilis L. and Zea mays L. plants.Water Air & Soil Pollution, 223, pp 5243–5257

Migliore, L., Cozzolino, S., Fiori, M. (2003). Phytotoxicity to and uptake of enrofloxacin in crop plants. Chemosphere, 52, pp 1233–1244.

Moldovan, Z., Chira, R., Alder, A.C. (2009). Environmental exposure of pharmaceuticals and musk fragrances in the Somes River before and arter upgrading the municipal wastewater treatment plant Cluj-Napoca, Romania. Environmental Science and Pollution Research, 16, pp 46–54.

Mollazadeh, N. (2014). Composting: a new method for reduction of solid waste and wastewater. International Research Journal of Applied and Basic Sciences, 8, pp 311–317

Mompelat, S., Le Bot, B., Thomas, O., (2009). Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water. Environment International, 35, pp 803–814.

Montforts, M.H.M.M. 2005. The trigger values in the environmental risk assessment for (veterinary) medicines in the European Union: a critical appraisal. RIVM report 601500002, p. 37

Morales-Toledo, A., Afonso-Olivares, C., Montesdeoca-Esponda, S., Guedes-Alonso, R., Sosa-Ferrera, Z., Santana-Rodriguez, J.J. (2016). Optimization and Development of SPE and MAE Combined with UHPLC-FD for the Determination of Acetylsalicylic Acid, Naproxen, Ibuprofen and Gemfibrozil in Sewage and Sludge Samples. Current Analytical Chemistry, 12, pp 545–552.

Mrozik, W. and Stefańska, J. (2014).Adsorption and biodegradation of antidiabetic pharmaceuticals in soils. *Chemosphere*, 95, pp 281–288.

Munir, M., Wong, K., Xagoraraki, I. (2011). Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. Water Research, 45, pp 681–693

Nafez, A.H., Nikaeen, M., Kadkhodaie, S., Hatamzadeh, M., Moghim, S. (2015). Sewage sludge composting: quality assessment for agricultural application. Environmental Monitoring and Assessment, 187, p. 9.

Naquin, A., Shrestha, A., Sherpa, M., Nathaniel, R., Boopathy, R. (2015). Presence of antibiotic resistance genes in a sewage treatment plant in Thibodaux, Louisiana, USA. Bioresource Technology, 188, pp 79–83.

Narumiya, M., Nakada, N., Yamashita, N., Tanaka, H. (2013). Phase distribution and removal of pharmaceuticals and personal care products during anaerobic sludge digestion. Journal of Hazardous Materials, 260, pp 305–312.

Nayak, A.K. and Kalamdhad, A.S. (2014). Feasibility of composting combinations of sewage sludge, cattle manure, and sawdust in a rotary drum reactor. Environmental Engineering Research, 19, p 47–57.

Nayak, A.K. and Kalamdhad, A.S. (2015). Sewage sludge composting in a rotary drum reactor: stability and kinetic analysis. International Journal of Recycling of Organic Waste in Agriculture, 4, pp 249–259.

Nei, L., Haiba, E., Kutti, S., Kipper, K., Herodes, K., Lillenberg, M. (2014). Sewage sludge compost, microbial activity and pharmaceuticals. Global Journal on Advances in Pure & Applied Sciences, 3, pp 30–37.

Nei, L., Haiba, E., Kutti, S., Lillenberg, M., Ivask, M., Peda, J., Kuu, A. (2015). Pharmaceuticals and the quality of sewage sludge compost. Abstracts Book: 2015 International Composting Conference. Beijing, China, p 14.

Nielsen, L. and Bandosz, T.J. (2016). Analysis of the competitive adsorption of pharmaceuticals on waste derived materials. Chemical Engineering Journal, 287, pp 139–147.

Niemuth, N.J. and Klaper, R.D. (2015). Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish. Chemosphere, 135, pp 38–45.

Nieto, A., Borrill, F., Pocurull, E., Marce, R.M.(2010). Occurrence of pharmaceuticals and hormoones in sewage sludge. Environmental Toxicology and Chemistry, 29, pp. 1484–1489,

Noirot-Cosson, P.E, Vaudour, E., Gilliot, J.M., Gabrielle, B., Houot, S. (2016). Modelling the long-term effect of urban waste compost applications on carbon and nitrogen dynamics in temperate cropland. Soil Biology and Biochemistry, 94, pp. 138–153.

Nouri, J., Mahvi, A.H., Jahed, G.R., Babaei, A.A. (2008). Regional distribution pattern of groundwater heavy metals resulting from agricultural activities. Environmental Geology, 55, pp 1337–1343.

Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout, B.A., Shivaprasad, H.L., Ahmed, S., Chaudhry, M.J.I., Arshad, M., Mahmood, S., Ali, A. Khan, A.A. (2004).Diclofenac residues as the cause of vulture population decline in Pakistan. Nature, 427, pp 630–3.

Olaniyan, L.W.B., Mkwetshana, N., Okoh, A.I. (2016). Triclosan in water, implications for human and environmental health. SpringerPlus, 5, 1639.

Oleszczuk, P. (2009). Sorption of phenanthrene by sewage sludge during composting in relation to potentially bioavailable contaminant content. Journal of Hazardous Materials, 161, pp 1330–1337.

Onwosi,C.O., Igbokwe, V.C., Odimba, J.N., Eke, I.E.,Nwankwoala, M.O., Iroh, I.N., Ezeogu, L.I. (2017). Composting technology in waste stabilization: On the methods, challenges and future prospects. Review. Journal of Environmental Management, 190, pp 140–157.

Oosterhuis, M., Sacher,F.L. ter Laak, T. (2013). Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data. Science of the Total Environment, 442, pp 380–388.

Osorio, V., Imbert-Bouchard, M., Zonja, B., Abad, J.L., Péreza, S., Barceló, D. (2014). Simultaneous determination of diclofenac, its human metabolites and microbial nitration/nitrosation transformation products in wastewaters by liquid chromatography/quadrupole-linear ion trap mass spectrometry. Journal of Chromatography A, 1347, pp 63–71.

Paltiel, O., Fedorova, G., Tadmor, G., Kleinstern, G., Maor, Y., Chefetz, B. (2016). Human exposure to wastewater-derived pharmaceuticals in fresh produce: a randomized controlled trial focusing on carbamazepine. Environmental Science & Technology, 50, pp 4476–4482.

Patureau, D., Hernandez-Raquet, G., Balaguer, P., Delgenes, N., Muller, M., Dagnino, S., Delgenes, J.P. (2008). Relevant approach to assess performances of wastewater biosolids composting in terms of micropollutants removal. Water Science & Technology, 58, pp 45–52.

Pérez, S., Eichhorn, P., Aga, D.S. (2005). Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment. Environmental Toxicology and Chemistry, 24, pp 1361–1367.

Peysson, W. and Vulliet, E. (2013). Determination of 136 pharmaceuticals and hormones in sewage sludge using quick, easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography–time-of-flight-mass spectrometry. Journal of Chromatography A, 1290, pp 46–61.

Pintado-Herrera, M.G., González-Mazo, E., Lara-Martín, P.A. (2014). Determining the distribution of triclosan and methyl triclosan in estuarine settings. Chemosphere, 95, pp 478–485.

Platen, H. and Wirtz, A. 1999. Application of analysis no 1: Measurement of the respiration activity of soils using the OxiTop® Control measuring system. Basic principles and process characteristic quantities. Wissenschaftlich- Technische Werkstätten GmbH & Co. Germany

Poulsen, T.G. and Bester, K. (2010). Organic micropollutant degradation in sewage sludge during composting under thermophilic conditions. Environmental Science & Technology, 44, pp 5086–5091.

Qiu, J., He, J., Liu, Q., Guo, Z., He, D., Wu, G., Xu, Z. (2012). Effects of conditioners on sulfonamides degradation during the aerobic composting of animal manures. Procedia Environmental Sciences, 16, pp 17–24.

Ramaswamy, J., Prasher, S.O., Patel, R.M., Hussain, S.A., Barrington ,S.F. (2010). The effect of composting on the degradation of a veterinary pharmaceutical. Bioresource Technology, 101, pp 2294–2299.

Redshaw, C.H., Wootton, V.G., Rowland, S.J. (2008). Uptake of the pharmaceutical Fluoxetine Hydrochloride from growth medium by Brassicaceae. Phytochemistry, 69, pp 2510–2516.

Reichel, R., Rosendahl, I., Peeters, E.T.H.M., Focks, A., Groeneweg J., Bierle, R., Schlichting, A., Amelung, W., Thiele-Bruhn, S. (2013). Effects of slurry from sulfadiazine-(SDZ) and difloxacin-(DIF) medicated pigs on the structural diversity of microorganisms in bulk and rhizosphere soil. Soil Biology and Biochemistry, 62, pp 82–91.

Reitman, M.L. and Schadt, E.E. (2007). Pharmacogenetics of metformin response: a step in the path toward personalized medicine. The Journal of Clinical Investigation, 117, pp 1226–1229.

Rezka, P., Balcerzak, W., Kwaśny, J. (2015). Carbamazepine and other anti-epileptic drugs in the aquatic environment. Technical Transactions, Environment Engineering, 1-Ś/2015, pp 111–118.

Ribas, J.L.C., da Silva, C.A., de Andrade, L., Galvan, G.L., Cestari, M.M., Trindade, E.S., Zampronio, A.R., de Assis H.C.A. (2014). Effects of anti-inflammatory drugs in primary kidney cell culture of a freshwater fish. Fish & Shellfish Immunology, 40, pp 296–303.

Richardson, M.L., and Bowron, J.M. (1985). The fate of pharmaceutical chemicals in the aquatic environment – a review. Journal of Pharmacy and Pharmacology, 37, pp 1–12.

Rivera-Utrilla, J., Sánchez-Polo, M., Ferro-García, M.A., Prados-Joya, G., Ocampo-Pérez, R. (2013). Pharmaceuticals as emerging contaminants and their removal from water. A review. Chemosphere, 93, pp 1268–1287.

Roasto, M., Praakle-Amin, K., Meremäe, K., Tamme, T., Hörman, A., Lillenberg, M., Hänninen, M-L. (2009). Food control and research on Campylobacter spp. in Estonia. Archiv für Lebensmittelhygiene, 60, pp 109–115.

Rodarte-Morales, A.I., Feijoo, G., Moreira, M.T., Lema, J.M. (2012). Biotransformation of three pharmaceutical active compounds by the fungus Phanerochaete chrysosporium in a fed batch stirred reactor under air and oxygen supply. Biodegradation, 23, pp 145–156

Rodríguez, L., Cerrillo, M.I., García-Albiach, V., Villaseñor, J. (2012). Domestic sewage sludge composting in a rotary drum reactor: Optimizing the thermophilic stage. Journal of Environmental Management, 112, pp 284–291.

Rodríguez-Rodríguez, C. E., Jelic´, A., Llorca, M., Farré, M., Caminal, G., Petrovic´, M., Barceló, D., Vicent. T. (2011). Solid-phase treatment with the fungus Trametes versicolor substantially redudes pharmaceutical concentrations and toxicity from sewage sludge. Bioresource Technology, 102, pp 5602–5608.

Rodríguez-Rodríguez, C.E., Barón,E., Gago-Ferrero, P., Jelic, A., Llorca, M., Farré, M., Díaz-Cruz, M.S., Eljarrat, E., Petrovic, M., Caminal, G., Barceló, D., Vicent, T. (2012). Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus Trametes versicolor in bioslurry reaktor. Journal of Hazardous Materials, 233– 234, pp 235–243.

RxList1. The Internet Drug Index. http://www.rxlist.com/cipro-drug.htm

RxList2. The Internet Drug Index. http://www.rxlist.com/noroxindrug.htm

RxList3. The Internet Drug Index. http://www.rxlist.com/floxin-drug.htm

RxList4. The Internet Drug Index.http://www.rxlist.com/carbatrol-drug.htm

Sadef, Y., Poulsen, T. G., Bester, K. (2014). Impact of compost process temperature on organic micro-pollutant degradation. Science of the total environment, 494-495, pp 306–312.

Sagristà, E., Larsson, E., Ezoddin, M., Hidalgo, M., Salvadó, V., Jönsson J.Å. (2010). Determination of non-steroidal anti-inflammatory drugs in sewage sludge by direct hollow fiber supported liquid membrane extraction and liquid chromatography–mass spectrometry. Journal of Chromatography A, 1217, pp 6153–6158.

Schaumann, R. and Rodloff, A.C. (2007). Activities of quinolones against obligately anaerobic bacteria. Anti-Infective Agents in Medicinal Chemistry, 6, pp 49–56.

Scheurer M., Sacher F., Brauch H.-J. (2009). Occurrence of the antidiabetic drug metformin in sewage and surface waters in Germany. Journal of Environmental Monitoring, 9, pp 1608–1613.

Shenker, M., Harush, D., Ben-Ari, J., Chefetz, B. (2011). Uptake of carbamazepine by cucumber plants– a case study related to irrigation with reclaimed wastewater. Chemosphere, 82, pp 905−910.

Singh, R.P. and Agrawal, M. (2010). Variations in heavy metal accumulation, growth and yield of rice plants grown at different sewage sludge amendment rates. Ecotoxicology and Environmental Safety, 7, pp 632–641.

Straub, J.O. (2016). Aquatic environmental risk assessment for human use of the old antibiotic sulfamethoxazole in Europe. Environmental Toxicology and Chemistry, 35, pp 767–779.

Stülten, D., Zühlke, S., Lamshöft, M., Spiteller, M. (2008).Occurrence of diclofenac and selected metabolites in sewage effluents. Science of the Total Environment, 405, pp 310–316.

Ternes, T.A., Joss, A., Siegrist, H., (2004). Scrutinizing pharmaceuticals and personal care products in wastewater treatment. Environmental Science and Technology. 38, pp 392A–399A.

Thiele-Bruhn S. (2003). Pharmaceutical antibiotic compounds in soils – a review. Journal of Plant Nutrition and Soil Science. 166, pp 145–167.

Thiele-Bruhn, S., Seibicke, T., Schulten, H.-R., Leinweber, P. (2004). Sorption of sulfonamide pharmaceutical antibiotics on whole soils and particle-size fractions. Journal of Environmental Quality, 33, pp 1331–1342.

Tohidi, F. and Cai, Z. (2017). Fate and mass balance of triclosan and its degradation products: comparison of three different types of wastewater treatments and aerobic/anaerobic sludge digestion. Journal of Hazardous Materials, 323, pp 329–340.

Trautwein,C. and Kümmerer, K. (2011). Incomplete aerobic degradation of the antidiabetic drug metformin and identification of the bacterial dead-end transformation product guanylurea. *Chemosphere*, 85, pp 765–773.

Trautwein, C., Berset, J-D., Wolschke, H., Kümmerer, K. (2014). Occurrence of the antidiabetic drug Metformin and its ultimate transformation product Guanylurea in several compartments of the aquatic cycle. Environment International, 70, pp 203–212

Tsigos, C., Hainer, V., Basdevant, A., Finer, N., Fried, M., Mathus-Vliegen, E., Micic D., Maislos, M., Roman, G., Schutz, Y. (2008). Management of obesity in adults: European clinical practice guidelines. Obesity facts. The European Journal of Obesity, pp 106–116.

Varnik, R., Karp, K., Pihelgas, E., Merivee, A., Lehtsaar, J. (2006). Effect of semicoke-based compost mixtures on gross margin of horticultural crops. Oil Shale, 23, pp 39–52.

Vasskog, T., Bergersen, O., Anderssen, T., Jensen, E., Eggen, T. (2009). Depletion of selective serotonin reuptake inhibitors during sewage sludge composting. Waste Management, 29, pp 2808–2815.

Verlicchi, P. and Zambello, E. (2016). Predicted and measured concentrations of pharmaceuticals in hospital effluents. Examination of the strengths and weaknesses of the two approaches through the analysis of a case study. Science of the Total Environment, 565, pp 82–94.

Verlicchi, P., Al Aukidy, M., Zambello, E., (2012). Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment—a review. Science of the Total Environment, 429, pp 123–155.

Walters, E., McClellan, K., Halden, R.U. (2010). Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. Water Research, 44, pp 6011–6020.

White, R.E., Torri, S.I. and Corrêa, R.S. (2011). Biosolids Soil Application: Agronomic and Environmental Implications. Applied and Environmental Soil Science, 2011, pp 1–3.

Winker, M., Clemens, J., Reich, M., Gulyas, H., Otterpohl, R. (2010). Ryegrass uptake of carbamazepine and ibuprofen applied by urine fertilization. Science of The Total Environment, 408, pp 1902–1908.

Winkler, M., Faika, D., Gulyas, H., Otterpohl, R. (2008). A comparison of human pharmaceutical concentrations in raw municipal wastewater and yellowwater. Science of the Total Environment, 399, pp 96–104.

Xu, J., Wu, L., Chang, A.C., (2009). Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils. *Chemosphere*, 77 (10), pp 1299–1305.

Yang S-F, Lin C-F, Lin AY-C, Hong P-KA. (2011). Sorption and biodegradation of sulphonamide antibiotics by activated sludge: experimental assessment using batch data obtained under aerobic conditions. Water Research, 45, pp 3389–3397.

Ying, G. G., and Kookana, R. S. (2007). Triclosan in wastewaters and biosolids from Australian wastewater treatment plants. Environment International, 33 (2), pp 199–205.

Ying, G. G., Kookana, R. S., Dillon, P. (2003). Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. Water Research, 37(15), pp 3785–3791.

Zhang, Y., Geißen, S-U., Gal, C. (2008). Review. Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies. Chemosphere, 73, pp 1151–1161.

Zhang, J., Chen, T-B, Gao, D. (2016). Simulation of the Mathematical Model of Composting Process of Sewage Sludge, Compost Science & Utilization, 24, pp 73–85.

Zhang, Y., Zhu, Y.G., Houot, S., Qiao, M., Nunan, N., Garnier, P. (2011). Remediation of polycyclic aromatic hydrocarbon (PAH) contaminated soil through composting with fresh organic wastes. Environmental Science and Pollution Research, 18, pp 1574–1584.

Zhou, H.-B., Ma, C., Gao, D., Chen, T.-D., Zheng, G.-D., Chen, J., Pan, T.-H. (2014). Application of a recyclable plastic bulking agent for sewage sludge composting. Bioresource Technology, 152, pp 329–336.

Zuloaga, O., Navarro, P., Bizkarguenaga, E., Iparraguirre, A., Vallejo, A., Olivares, M., Prieto, A. (2012). Overview of extraction, clean-up and detection techniques for the determination of organic pollutants in sewage sludge: a review. Analytica Chimica Acta, 736, pp 7–29.

ACKNOWLEDGMENTS

First of all, I am grateful to AS Tallinna Vesi, AS Viljandi Veevärk and AS Epler & Lorenz for their cooperation and valuable assistance.

I would like to express my deepest gratitude to my supervisor Prof. Lembit Nei for the guidance, support and encouragement during my PhD studies. I would not have been able to complete my doctoral thesis without his thorough knowledge, timely suggestions, inspiring and enlightening discussions. Likewise, I would like to thank my second supervisor, Dr Merike Lillenberg, for her advice, support, and for sharing her academic knowledge with me throughout our collaboration over the years of my studies.

It is my privilege to thank the chemists from the University of Tartu, Institute of Chemistry, Dr Karin Kipper and Dr Koit Herodes, for helping me with the experiments, measurements and guidance. I appreciate MSc Sander Kutti and MSc Jane Raamets for the discussions, help, advice and cooperation with the experiments and writing the publications.

My sincere thanks to my wonderful colleagues at Tartu College, Tallinn University of Technology, Kaie Lehtme, Annely Kuu, Aime Ruus, Ago Rootsi, Tiina Niine and Helle Hallik for the countless times they provided me with necessary advice, technical suggestions and good company during my research. I would like to thank Prof. Mari Ivask and Karin Mouni for their continuous motivation, support and advises.

Finally, I would like to express my heartfelt thanks to my Kristjan, family and friends for sharing my high and low moments. My warmest gratitude belongs to my mother Eiri and aunt Virge for their love, continuous support and patience. Without them believing in me, it would be much harder to achieve my goals.

ABSTRACT

Optimization of sewage sludge composting: problems and solutions

Composting is one of the sustainable practices to convert sewage sludge into useful agricultural product. Before the compost can be used for agricultural purposes, international and existing regulations in Estonia foresee its analysis for heavy metals, coliform-like bacteria and helminth eggs; however, there is no requirement for the determination of the content of organic toxic compounds in it. During the last decade, scientists have become keenly aware that toxic compounds present in sewage sludge are dangerous to the environment. Sewage sludge may contain many pharmaceutical residues and other toxic compounds, some of which are not biodegradable. So with sewage sludge compost, they may get into the field, where they can change the biological balance of soil and accumulate in food plants. Even extra-low concentrations of pharmaceutical residues and toxic compounds can endanger ecological balance and human health. Although, it is known that sewage sludge composting can induce the degradation of toxic compounds in it, the effect of sewage sludge composting technologies on the degradation of pharmaceutical residues has not been systematically studied.

The aim of this work was to study the impact of sewage sludge composting on the degradation of some widely used pharmaceuticals and to add a piece of knowledge applicable in the development of composting technologies leading to the increase of the safety of using sewage sludge compost in the fertilization of soils with poor nutrient content. The main directions of the research presented in this thesis were:

- The pilot studies and literature analysis showed that sewage sludge contains a high variety of pharmaceutical residues that are hazardous to the environment and that some of these residues can be transported to food plants via the usage of sewage sludge or its compost as fertilizers.
- The possibilities of reducing the impact of pharmaceuticals on the environment and people through increasing the efficiency of sewage sludge composting technologies were studied.
- Efficient sewage sludge composting conditions with the aim of achieving more complete degradation of FQ and SA residues were developed.
- The impact of bulking agent on the degradation of DCF, MET, CBZ and TCS residues in sewage sludge compost were determined and an optimal compost mixture composition was proposed.
- Recommendations directed to reducing environmental pollution resulting from pharmaceutical residues present in sewage sludge.

The results of this study showed that in the case of several pharmaceuticals it is possible to enhance their degradation rate through the intelligent selection of sewage sludge composting conditions. Additions of sawdust clearly speeded up this process, whereas the mixtures with peat and straw perform lower abilities to

decompose pharmaceutical residues. The results of this study showed that the optimization of composting technologies (higher C-content as a bulking agent) allowed to carry out the efficient degradation of DCF, MET and TCS, while for the elimination of CBZ from sewage sludge different means should be used. The composting period should last for at least six months.

Although sewage sludge composting is an efficient way for degrading several widely used pharmaceuticals, some of these compounds are highly persistent towards bioprocesses and their elimination needs the application of different measures. At the moment, little is known about the long-term use of sewage sludge and its compost and how the presence of contaminants and in particular their mixtures affect soil micro-organisms, and therefore work in this area should continue.

KOKKUVÕTE

Reoveesette kompostimistehnoloogiate optimeerimine keskkonnaohutuse nõuetest lähtuvalt

Reoveesete on toitaineterikas substraat, kuid tema kasutamine mullaviljakuse tõstjana on piiratud seetõttu, et ta sisaldab paljusid keskkonda reostavaid ühendeid, sealhulgas ravimite jääke. Kuigi reoveesette kompostimisel paljud orgaanilised ühendid lagunevad, siis osade laialdast kasutamist leidvate ravimijääkide lagunemise kiirus ei ole küllaldane. Murettekitavaks on muutunud ravimijääkide potentsiaalne migreerumine toidutaimedesse. Erinevate saasteainete jääkide sattumine pinnasesse võib mõjutada taimede kasvu ja arengut ning samuti komposti bakterite ja seente elutegevust ning aktiivsust. Eesti suuremates veepuhastusjaamades kasutatavad kompostimistehnoloogiad vajavad optimeerimist, et tagada toiduohutuse seisukohalt hädavajalik saasteainete võimalikult madal sisaldus väetamiseks kasutatavas kompostis. Käesoleva väitekirja tulemused võimaldavadki selles suunas edasi liikuda.

Käesoleva töö eesmärgiks oli uurida reoveesette kompostimistehnoloogiate mõju mõnede laialdaselt kasutatavate farmaatsiatoodete jääkide lagunemisele, mille tulemusena oleks võimalik laiendada reoveesette komposti ohutut kasutamist toitainetevaeste muldade väetamisel. Püstitatud ülesande lahendamisel läbiti järgmised etapid:

- Eelkatsete ja kirjanduse analüüsi tulemusena näidati, et reoveesete sisaldab keskkonnaohtlikkuse seisukohalt olulistes kogustes laialdast kasutust leidvate ravimite jääke ning et osad nendest ravimitest võivad reoveesette või tema komposti kasutamisel liikuda toidutaimedesse.
- Uuriti võimalusi mõningate laialdaselt kasutatavate farmaatsiatoodete mõju vähendamiseks keskkonnale ja inimestele reoveesette kompostimistehnoloogiate tõhustamise abil.
- Töötati välja fluorokinoloonide (FQ) ja sulfoonamiidide (SA) jääkide kiiremat ja täielikumat lagunemist soodustavad kompostimistingimused.
- Määrati sette ja tugimaterjali koostise mõju diklofenaki, metformiini, karbamasepiini ja triklosaani lagunemisele reoveesette kompostis ning pakuti välja Eesti tingimustele vastav optimaalne kompostisegu koostis.
- Esitati reoveesettes sisalduvatest ravimijääkidest tuleneva keskkonnaohtlikkuse kahandamisele suunatud soovitused.

Läbiviidud uurimuse tulemused näitasid, et optimaalsete kompostimis-tehnoloogiate teel on võimalik paljude saasteainete sisaldust reoveesette kompostis olulisel määral vähendada. Hetkel on vähe teada selle kohta, kuidas reoveesette ja komposti pikaajaline kasutamine ja seal leiduvad saasteained ning eriti nende segud mõjutavad mulla mikroorganisme, mistõttu töö selles valdkonnas peaks jätkuma.

APPENDIX A

PAPER I

Haiba, E., Nei, L., Kutti, S., Lillenberg, M., Herodes, K., Ivask, M., Kipper, K., Aro, R., Laaniste, A. (2017). Degradation of diclofenac and triclosan residues in sewage sludge compost. *Agronomy Research*, 15 (2), 395−405.

Agronomy Research **15**(2), 395–405, 2017

Degradation of diclofenac and triclosan residues in sewage sludge compost

E. Haiba[1], L. Nei[1,*], S. Kutti[1], M. Lillenberg[2], K. Herodes[3], M. Ivask[1], K. Kipper[3], R. Aro[3] and A. Laaniste[3]

[1]Tartu College, Tallinn University of Technology, Puiestee 78, EE51008 Tartu, Estonia
[2]Estonian University of Life Sciences, Kreutzwaldi 58A, EE51014 Tartu, Estonia
[3]Institute of Chemistry, University of Tartu, Ravila 14A, EE51010 Tartu, Estonia
[*]Correspondence: lembit.nei@ttu.ee

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.

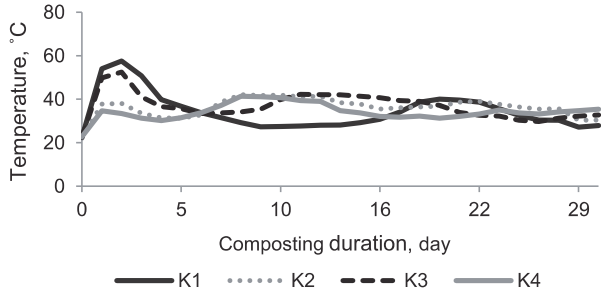

Figure 1. Temperature profiles during one month composting for 1:2 (v:v) sewage sludge – sawdust mixtures: K1 – containing pharmaceuticals; K2 – without pharmaceuticals and for 1:3 (v:v) sewage sludge – sawdust mixtures: K3 – containing pharmaceuticals; K4 – without pharmaceuticals.

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.

ACKNOWLEDGEMENTS. The authors would like to thank Environmental Investment Centre of Estonia for funding this work.

REFERENCES

Al-Rajab, A.J., Sabourin, L., Lapen, D.R. & Topp, E. 2010. The non-steroidal anti-inflammatory drug diclofenac is readily biodegradable in agricultural soils. *Science of the Total Environment* **409**, 78–82.

Aydin, S., Ince, B. & Ince, O. 2015. Development of antibiotic resistance genes in microbial communities during long-term operation of anaerobic reactors in the treatment of pharmaceutical wastewater. *Water Research* **83**, 337–344.

Baharoglu, Z. & Mazel, D. 2011. Vibrio cholerae triggers SOS and mutagenesis in response to a wide range of antibiotics: a route towards multiresistance. *Antimicrobial Agents and Chemotheraphy* **55**, 2438–2441.

Banegas, V., Moreno, J.L., Moreno, J.I., García, C., León, G. & Hernández, T. 2007. Composting anaerobic and aerobic sewage sludges using two proportions of sawdust, *Waste Management* **27**, 1317–1327.

Baquero, F., Martinez, J.L. & Canton, R. 2008. Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology* **19**, 260–265.

Bartha, B., Huber, C. & Schröder, P. 2014. Uptake and metabolism of diclofenac in Typha latifolia – How plants cope with human pharmaceutical pollution. *Plant Science* **227**, 12–20.

Butkovskyi, A., Ni, G., Leal Hernandez, L., Rijnaarts, H.H.M. & Zeeman, G. 2016. Mitigation of micropollutants for black water application in agriculture via composting of anaerobic sludge. *Journal of Hazardous Materials* **303**, 41–47.

Casado, J., Castro, G., Rodríguez, I., Ramil, M. & Cela, R. 2015. Selective extraction of antimycotic drugs from sludge samples using matrix solid-phase dispersion followed by on-line clean-up. *Analytical and Bioanalytical Chemistry* **407**, 907–917.

Chen, G., den Braver, M.W., van Gestel C.A.M, van Straalen N.M. & Roelofs, D. 2015. Ecotoxicogenomic assessment of diclofenac toxicity in soil. *Environmental Pollution* **199**, 253–260.

Chen, X., Nielsen, J.L., Furgal, K., Liu, Y., Lolas, I.H. & Bester, K. 2011. Biodegradation of triclosan and formation of methyl-triclosan in activated sludge under aerobic conditions. *Chemosphere* **84**, 452–456.

Chroni, C., Kyriacou, A., Manios, T. & Lasaridi, K-E. 2009. Investigation of the microbial community structure and activity as indicators of compost stability and composting process evolution. *Bioresource Technology* **100**, 3745–3750.

Dobor, J., Varga M., Yao J., Chen H., Palkó G. & Záray G. 2010. A new sample preparation method for determination of acidic drugs in sewage sludge applying microwave assisted solvent extraction followed by gas chromatography–mass spectrometry. *Microchemical Journal* **94**, 36–41.

Garcia-Rodriguez, A., Sagrista, E., Matamoros, V., Fontas, C., Hidalgo, M. & Salvado, V. 2014. Determination of pharmaceutical compounds in sewage sludge using a standard addition method approach. *International Journal of Environmental Analytical Chemistry* **94**, 1199–1209.

Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C. & Giger, W. 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Environmental Science & Technology* **37**, 3243–3249.

Göbel, A., Thomsen, A., McArdell, C.S., Joss, A. & Giger, W. 2005. Occurrence and Sorption Behavior of Sulfonamides, Macrolides, and Trimethoprim in Activated Sludge Treatment. *Environmental Science & Technology* **39**, 3981–3989.

Haiba, E., Nei, L., Ivask, M., Peda, J., Järvis, J., Lillenberg, M., Kipper, K. & Herodes, K. 2016. Sewage sludge composting and fate of pharmaceutical residues – recent studies in Estonia. *Agronomy Research* **14,** 1583−1600.

Haiba, E., Nei, L., Lillenberg, M., Kipper, K. & Herodes K. 2013. Degradation of some pharmaceuticals during sewage sludge composting. *Global Journal on Advances in Pure & Applied Sciences* **1**, 827–832.

Huber, C., Bartha, B. & Schröder, P. 2012. Metabolism of diclofenac in plants – Hydroxylation is followed by glucose conjugation. *Journal of Hazardous Materials* **243**, 250– 256.

Jelić, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R.,Ventura, F., Petrovic, M. & Barcelo D. 2011. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. *Water Research* **45**, 1165–1176.

Jelić, A., Petrović, M. & Barcelóa D. 2009. Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry. *Talanta* **80**, 363–371.

Järvis, J., Ivask, I., Nei, L., Kuu, A. & Luud, A. 2016. Effect of Green Waste Compost Application on *Afforestation Success. Baltic Forestry* **22**, 90−97.

Kalda, K., Ivask, M., Kutti, S., Kuu, A., Meriste, M., Nei, L., Peda, J. & Raukas, A. 2015. Soil Invertebrates in Semi-Coke Heaps of Estonian Oil Shale Industry. *Oil Shale* **32**, 82−97.

Kaonga, C.C., Kumwenda, J. & Mapoma, H.T. 2010. Accumulation of lead, cadmium, manganese, copper and zinc by sludge worms; *Tubifex tubifex* in sewage sludge. *International Journal of Environmental Science* and *Technology* **7**, 119–126.

Kim, K.-R., Owens, G., Ok, Y.S, Park, W.-K., Lee, D.B. & Kwon, S.-I. 2012 Decline in extractable antibiotics in manure-based composts during composting. *Waste Management* **32**, 110–116.

Kipper, K., Herodes, K., Lillenberg, M., Nei, L., Haiba, E. & Litvin, S.V. 2010. Plant uptake of some pharmaceuticals commonly present in sewage sludge compost. *In proceedings of 2nd International Conference on Chemical, Biological and Environmental Engineering, Cairo, Egypt, 2-4 November 2010,* 261–264.

Kipper, K., Herodes, K., Leito, I. & Nei, L. 2011. Two fluoroalcohols as components of basic buffers for liquid chromatography electrospray ionization mass spectrometric determination of antibiotic residues. *Analyst* **136**, 4587−4594.

Li, Y., Zhu, G., Jern Ng, W. & Tan, S.K. 2014. A review on removing pharmaceutical contaminants from waste water by constructed wetlands: Design, performance and mechanism. *Science of the Total Environment* **468–469**, 908–932.

Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Lõhmus, R., Ivask, M., Kuu, A., Kutti, S., Litvin, S.V. & Nei, L. 2010. Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting. *International Journal of Environmental Science and Technology* **7**, 307−312.

Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Sepp, K., Lõhmus, R. & Nei, L. 2009. Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry. *Journal of Chromatography A* **1216**, 5949−5954.

Loos, R., Carvalho, R., António, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D. & Gawlik, B.M. 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Research* **47**, 6475–6487.

Lozano, N., Rice, C.P., Ramirez, M. & Torrents, A. 2010. Fate of triclosan in agricultural soils after biosolid applications. *Chemosphere* **78**, 760–766.

Morales-Toledo, A., Afonso-Olivares, C., Montesdeoca-Esponda, S., Guedes-Alonso, R., Sosa-Ferrera, Z. & Santana-Rodriguez, J.J. 2016. Optimization and Development of SPE and MAE Combined with UHPLC-FD for the Determination of Acetylsalicylic Acid, Naproxen, Ibuprofen and Gemfibrozil in Sewage and Sludge Samples. *Current Analytical Chemistry* **12**, 545–552.

Munir, M., Wong, K. & Xagoraraki, I. 2011. Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan.*Water Research* **45**, 681–693.

Mäesaar, M., Kramarenko, T., Meremäe, K., Sõgel, J., Lillenberg, M., Häkkinen, L., Ivanova, M., Kovalenko, K., Hõrman, A., Hänninen, M-L. & Roasto, M. 2016. Antimicrobial resistance profiles of *Campylobacter spp.* isolated from broiler chicken meat of Estonian, Latvian and Lithuanian origin at Estonian retail level and from patients with severe enteric infections in Estonia. *Zoonoses Public Health* **63**, 89−96.

Naquin, A., Shrestha, A., Sherpa, M., Nathaniel, R. & Boopathy, R. 2015. Presence of antibiotic resistance genes in a sewage treatment plant in Thibodaux, Louisiana, USA. *Bioresource Technology* **188**, 79–83.

Nei, L., Haiba, E., Kutti, S., Kipper, K., Herodes, K. & Lillenberg, M. 2014. Sewage sludge compost, microbial activity and pharmaceuticals. *Global Journal on Advances in Pure & Applied Sciences* **3**, 30–37.

Nei, L., Haiba, E., Kutti, S., Lillenberg, M., Ivask, M., Peda, J. & Kuu, A. 2015. Pharmaceuticals and the quality of sewage sludge compost. *Abstracts Book: 2015 International Composting Conference*. Beijing, China, 14.

Osorio, V., Imbert-Bouchard, M., Zonja, B., Abad, J.L., Péreza, S. & Barceló, D. 2014. Simultaneous determination of diclofenac, its human metabolites and microbial nitration/nitrosation transformation products in wastewaters by liquid chromatography/quadrupole-linear ion trap mass spectrometry. *Journal of Chromatography A* **1347**, 63–71.

Pintado-Herrera, M.G., González-Mazo, E. & Lara-Martín, P.A. 2014. Determining the distribution of triclosan and methyl triclosan in estuarine settings. *Chemosphere* **95**, 478–485.

Platen, H. & Wirtz, A. 1999. Application of analysis no 1: Measurement of the respiration activity of soils using the OxiTop® Control measuring system. *Basic principles and process characteristic quantities.* Wissenschaftlich- Technische Werkstätten GmbH & Co. Germany

Poulsen, T.G. & Bester, K. 2010. Organic micropollutant degradation in sewage sludge during composting under thermophilic conditions. *Environmental Science & Technology* **44**, 5086–5091.

Ribas, J.L.C., da Silva, C.A., de Andrade, L., Galvan, G.L., Cestari, M.M., Trindade, E.S., Zampronio, A.R. & de Assis, H.C.A. 2014. Effects of anti-inflammatory drugs in primary kidney cell culture of a freshwater fish. *Fish & Shellfish Immunology* **40**, 296–303.

Roasto, M., Praakle-Amin, K., Meremäe, K., Tamme, T., Hörman, A., Lillenberg, M., Hänninen, M-L. 2009. Food control and research on *Campylobacter spp.* in Estonia. *Archiv für Lebensmittelhygiene* **60**, 109–115.

Sagristà, E., Larsson, E., Ezoddin, M., Hidalgo, M., Salvadó, V. & Jönsson, J.Å. 2010. Determination of non-steroidal anti-inflammatory drugs in sewage sludge by direct hollow fiber supported liquid membrane extraction and liquid chromatography–mass spectrometry. *Journal of Chromatography A* **1217**, 6153–6158.

Stülten, D., Zühlke, S., Lamshöft, M. & Spiteller, M. 2008. Occurrence of diclofenac and selected metabolites in sewage effluents. *Science of The Total Environment* **405**, 310–316.

Zhang, Y., Geißen, S-U. & Gal, C. 2008. Review. Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere* **73**, 1151–1161.

Zhang, Y., Zhu, Y.G., Houot, S., Qiao, M., Nunan, N. & Garnier, P. 2011. Remediation of polycyclic aromatic hydrocarbon (PAH) contaminated soil through composting with fresh organic wastes. *Environmental Science and Pollution Research* **18**, 1574–1584.

Yang, S.-F., Lin, C.-F., Lin, A.Y.-C. & Hong, P.-K.A. 2011. Sorption and biodegradation of sulfonamide antibiotics by activated sludge: Experimental assessment using batch data obtained under aerobic conditions. *Water Research* **45**, 3389–3397.

PAPER II

Kipper, K., Lillenberg, M., Herodes, K., Nei, L., **Haiba, E**. (2017). Simultaneous determination of fluoroquinolones and sulfonamides originating from sewage sludge compost. *The Scientific World Journal*, 2017, 8p.

Hindawi
The Scientific World Journal
Volume 2017, Article ID 9254072, 8 pages
https://doi.org/10.1155/2017/9254072

Research Article

Simultaneous Determination of Fluoroquinolones and Sulfonamides Originating from Sewage Sludge Compost

K. Kipper,[1] M. Lillenberg,[2] K. Herodes,[1] L. Nei,[3] and E. Haiba[3]

[1]*Institute of Chemistry, University of Tartu, Tartu, Estonia*
[2]*Estonian University of Life Sciences, Tartu, Estonia*
[3]*Tartu College, Tallinn University of Technology, Tartu, Estonia*

Correspondence should be addressed to K. Kipper; karin.kipper@gmail.com

Received 20 February 2017; Revised 1 May 2017; Accepted 11 May 2017; Published 12 June 2017

Academic Editor: Sandra Babic

A simultaneous method for quantitative determination of traces of fluoroquinolones (FQs) and sulfonamides (SAs) in edible plants fertilized with sewage sludge was developed. The compounds were extracted from the plants by rapid and simple liquid extraction followed by extracts clean-up using solid phase extraction. The eluent additive 1,1,1,3,3,3-hexafluoro-2-propanol was used for liquid chromatographic detection to achieve separation of structurally similar antimicrobials like ciprofloxacin and norfloxacin. Identification and quantification of the compounds were performed using high-performance liquid chromatography with electrospray ionization mass spectrometry in selected reaction monitoring mode. Method was validated and extraction recoveries of FQs and SAs ranged from 66% to 93%. The limit of quantifications was from 5 ng/g in the case of ofloxacin to 40 ng/g for norfloxacin. The method precision ranged from 1.43% to 2.61%. The developed novel method was used to evaluate the plats antimicrobial uptake (potato *(Solanum tuberosum* L.*)*, carrot *(Daucus carota* L.*)*, lettuce *(Lactuca sativa* L.*)*, and wheat *(Triticum vulgare* L.*)*) from soil and migration of the analytes inside the plants.

1. Introduction

The increase of the yearly production of sewage sludge compost containing human and veterinary antimicrobials has led to antimicrobial resistance being one of the top health challenges in the 21st century [1]. One of the largest and most diverse microbial habitats on Earth is soil, a vast repository of the antimicrobial resistance genes between soil bacteria and clinical pathogens [2].

When antimicrobials are eliminated from the human body, they can be excreted in their native form or as metabolites [3]. Since antimicrobials are developed to have a specific mode of action, even low levels of these drugs in edible plants can cause effects in organisms [4].

Several studies have demonstrated that the two most important sources through which toxic compounds reach the environment are sewage sludge and compost, which are often used in agriculture [5–9]. More generally, pharmaceuticals move into the sewage system and to waste water treatment plants [10]. The nutrition-rich sewage sludge and compost can be used as fertilizers for plants. The increasing proportions of administered drugs and personal care products are alarming because the compound releases into the environment are not controlled [11, 12] and this is a potential threat to the environment [13–15]. It is worrisome that pharmaceutical compounds may potentially enter edible food plants that have been fertilized with sewage sludge compost [9, 16–18].

The risks from the fertilizer should be evaluated carefully. Exposure to pharmaceuticals via plant-derived foodstuffs is usually low and effects on human health are in most cases unlikely. This route of exposure may, however, be more significant for a small number of highly toxic medicines or in situations where long-term low-level exposure could elicit subtler effects (e.g., promotion of antibacterial resistance or endocrine disruption) [19]. A chemical can undergo various structural changes by a multitude of biotic and nonbiotic processes after its introduction into the environment. Structural transformations may also be a result of effluent treatment [4, 20–26]. The maximum residue levels (MRL) are set only for food of animal origin, milk and meat [27, 28].

Analytical methods have been developed and applied for the determination of different antimicrobials in sewage sludge and its compost, biosolids, and sludge-treated soil [29–38]. Many antimicrobials, known to be persistent in soils fertilized with sewage sludge compost, can accumulate into food plants [39–46]. The pharmaceuticals accumulated in the food plants may generate resistant bacteria in human and animal organisms. The groups of antimicrobials of interest are well-known, but it is a complicated task to separate structurally similar compounds in a reversed-phase LC (liquid chromatography) system. On the other hand, the concentrations in residue levels are very low and therefore improved MS (mass spectrometry) sensitivity is more than welcome. The aim of the present study was to use an eluent additive 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) to improve LC separation significantly with alternative selectivity in C18 stationary phase and enhance MS detection of fluoroquinolones (FQs) and sulfonamides (SAs) in small concentration levels to quantify them in food plant samples. These drugs were selected according to three criteria: (1) their stability in soil [47], (2) their potential to accumulate into plants [39, 46], and (3) their presence in sewage sludge and its compost [48].

For the prevention of the development of microbial resistance of humans and animals, the concentration of antimicrobials in compost must be significantly below 1 μg/kg, securing the relevant soil concentrations at 0.01–0.1 μg/kg [49]. In our previous work [16], the highest detected concentrations of the antimicrobial norfloxacin (NOR), ciprofloxacin (CIP), ofloxacin (OFL), sulfamethoxazole (SMX), and sulfadimethoxine (SDM) in sewage sludge and its compost were as shown in Table 1.

Table 1: Occurrence of antimicrobials (μg/kg) in sewage sludge and its compost (illustrative data).

Antimicrobial media	NOR	CIP	OFL	SMX	SDM
Sewage sludge	162	426	39	6	20
Compost	22	20	3	1	4

2. Materials and Methods

2.1. Chemicals. Pharmaceuticals 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%). Acetonitrile and methanol were obtained from J.T. Baker (Deventer, Netherlands), HPLC grade formic acid, and ammonia from Riedel-de-Haën. HFIP was purchased from Sigma (St. Louis, MO, USA). All solvents were of reagent grade or higher quality. Water was purified (18.2 MΩ × cm at 25°C and a TOC value below 3 ppb) in-house using a Milli-Q Plus system from Millipore (Bedford, USA). Hydrophilic-lipophilic balanced (HLB) solid phase extraction (SPE) cartridges (Oasis HLB (60 μm), 500 mg/6 mL) were purchased from Waters (Milford, MA, USA).

The selection of antimicrobials was made according to preliminary pilot study on antimicrobials usage and presence in the sewage sludge samples [48], their stability in the soil and potential degradation during the composting procedure [16], and their uptake from the soil by plants [46].

2.2. Plant Samples. For the experiments with plants, the antimicrobials were spiked into the soil in which the plants were grown [49]. Aqueous solutions of the studied pharmaceuticals were mixed with soil. The final concentration of each pharmaceutical was 10 mg per kg of dry soil. To ensure better dissolution of the studied pharmaceuticals, fluoroquinolones were dissolved in 2 ml of 0.1 mM ammonium acetate buffer solution with pH 2.8 and sulfonamides were dissolved in 2 ml of 0.3 M NaOH. Potatoes *(Solanum tuberosum* L.*)*, carrots *(Daucus carota* L.*)*, lettuce *(Lactuca sativa* L.*)*, and wheat *(Triticum vulgare* L.*)* were grown in the presence of five antimicrobials commonly present in sewage sludge (CIP, NOR, OFL, SDM, and SMX). The potato tubers and plant seeds were planted into the pots, with one tuber or 35 seeds in each pot. The plants were cultivated in a greenhouse under natural light conditions for 120 days after planting (lettuce, 70 days). The soil was weighed, and aqueous solutions of the studied pharmaceuticals were mixed with the soil. The final concentration of each pharmaceutical was 0.01, 0.1, 0.5, 1, and 10 mg/kg (dry weight). Three parallel experiments were conducted for each concentration of antimicrobials. In reference experiments, plants were cultivated in antimicrobial-free soils. The plants were collected and washed carefully. Potatoes and carrots were chopped into ca 1 cm^3 pieces. Then the plants were dried at room temperature in the darkness and after that milled for analyses, using Knifetec 1095 Sample Mill (Foss) and a common coffee mill. The size of the particles of the powder was <1 mm^3. The milled samples were dewatered in a thermostat at 45°C for 24 hours using thermostat Binder KB 115 and stored in hermetic plastic bags for three weeks at −80°C (using refrigerator Sanyo MDF-U54V) before analysis.

2.3. Sample Preparation. 250 mg of dried plants (grains, roots, or leaves) was extracted with 10 mL of a 1 : 1 (v/v) mixture of acetonitrile and 1% acetic acid, then homogenized with laboratory homogenizer DIAX 900 (Heidolph Instruments, Germany) at 25,000 rpm, sonicated (5′), vortexed (1′), and centrifuged at 8000 rpm. The supernatant was then separated and dried by nitrogen stream to remove acetonitrile. Approximately 15 mL of 1% acetic acid was added to the 1 mL of evaporation residue [49].

The extract collected with liquid extraction was cleaned up with solid phase extraction (SPE). Antimicrobials, CIP, NOR, OFL, SDM, and SMX, were extracted using HLB cartridges. For the SPE procedure, the vacuum manifold (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 the cartridges using 12 mL of methanol. The SPE extracts were evaporated to dryness in polypropylene vials in an N2 stream. Residue was dissolved in 1 mL of 20% methanol with buffer solution (5 mM 1,1,1,3,3,3-hexafluoro-2-propanol, pH adjusted to 9.0 with NH_4OH).

2.4. Liquid Chromatography-Mass Spectrometry. Chromatographic separation of the analytes was carried out on the Agilent Series 1100 LC-MSD Trap XCT (Agilent Technologies, Santa-Clara, CA, USA) equipped with a binary pump, a degasser, an autosampler, and a column thermostat. Five antimicrobials were chromatographed using a Waters XBridge C18 column (150 mm × 3 mm, 3.5 μm) equipped with a Waters Guard Cartridge (20 mm × 4.6 mm) (Waters, Milford, USA). For detection, a diode array detector and ESI-MS were used in series. ESI-MS detection was carried out in positive ion detection mode. Selected reaction monitoring was used. Full MS^2 spectra were recorded and the following transitions were applied for quantification: OFL m/z 362→261, 318; NOR m/z 320→302, 276; CIP m/z 332→288, 314; SMX m/z 254→108, 188; SDM m/z 311→108, 156, 218, 245. Default parameters for ESI and MS were used for all the experiments (nebulizer gas pressure was 40 psi, dry gas flow was 10 L/min, dry gas temperature was 350°C, capillary voltage was 5000 V, detected mass range was from m/z 100 to 1000, and target mass for compounds was m/z 350). The LC-MS instrument was controlled by Agilent Chemstation for LC 3D rev. A.10.02 (Agilent Technologies) and LC/MSD Trap Control ver. 5.2 (Bruker Daltonik GmbH, Germany). Data analysis was carried out using Chemstation software (Agilent Technologies) and Data Analysis for LC/MSD Trap Version 3.2 (Bruker Daltonik GmbH).

2.5. Chromatographic Conditions. 5 mM HFIP buffer (pH adjusted with NH_4OH to 9.0) and methanol were used for elution. Gradient elution at flow rate 0.3 mL/min started at 10% methanol and was raised to 55% within 25 min, after which methanol concentration was raised to 100% within 5 min. Methanol concentration was kept at 100% for 5 min, then lowered to 10% in 5 min, and equilibrated at 10% for 5 min. Column temperature was set to 30°C and the injection volume was 10 μL.

2.6. Standard and Buffer Solutions. Stock solutions of the analytes at 1 mg/mL in the appropriate solvent (mixture of MeOH and 1 mM ammonium acetate buffer with 0.1% formic acid, 20/80) were prepared. The stock solution for SDM was 0.5 mg/mL due to its poor solubility. The working standard solution contained 5 antimicrobials at 0.1 mg/mL. From this solution dilution (10 μg/mL and 1 μg/mL) was made. The stock solution was stored at −20°C. Fresh working standard solutions were prepared daily.

2.7. Method Validation. The developed method was validated following Eurachem guidelines [50] and the linearity, limit of quantification, and process efficiency (recovery and matrix effect) were evaluated.

3. Results and Discussion

3.1. Liquid Extraction. The developed method is based on the combination of liquid extraction, SPE, and LC-MS analysis of a total of five antimicrobials. Antimicrobials from two classes, FQs and SAs, are structurally and chemically diverse. The variables optimized were extraction solvent, pH, and homogenization. Hexane, chloroform, methanol, and acetonitrile were tested as extraction solvents. The organic solvent content in an extraction solvent was varied from 20 to 100%. Extraction with chloroform and hexane gave the lowest overall antibiotic recoveries (1-2%) for CIP and NOR. The extraction mixture's aqueous solution's pH varied from acidic (1% acetic acid, pH 2.0) to basic (5 mM ammonium acetate, pH 9.0 (using NH_4OH)) conditions. Extraction with acetonitrile was more efficient compared with methanol. The mixture of acetonitrile and 1% acetic acid (1/1) was finally chosen as an extraction solvent for simultaneous extraction of all the analytes of interest. During the optimization of liquid extraction, it was found that extraction efficiency increased when homogenization was used along with sonication and mixing. The increase of the time of liquid extraction stages did not increase extraction recoveries. In total, the time for a LE procedure was 17 minutes.

3.2. Solid Phase Extraction (SPE). After liquid extraction and centrifugation, the supernatant was separated and dried by nitrogen stream to remove acetonitrile. Remaining extracts were cleaned up with HLB SPE cartridges. The HLB cartridges enable retaining both hydrophilic and hydrophobic compounds [51] and give the highest recoveries for all of the analytes studied. The sample pH was adjusted by adding approximately 15 mL of 1% acetic acid. For elution, methanol was used.

3.3. LC-MS. Previously several buffer solutions and pH values were tested thoroughly for the reverse phase (RP) LC separation of named antibiotics [31]. Although the MS signal of analytes was higher using an eluent with a lower pH (ammonium acetate, formic acid with pH 2.8), the separation of the compound was not followed. At higher pH values, the fluoroalcohol HFIP gave significantly better ionization efficiencies and better peak shapes, compared to acidic conditions and known buffer additives [47], and all 5 compounds had baseline separation for an antibiotic standard solution using RP column. For the LC analysis, the 5 mM HFIP and gradient elution with methanol was used. The application demonstrates the successful separation of chosen compounds from the potato tubers extract (Figure 1).

HFIP as an eluent additive is predominantly protonated at pH 9.0. Therefore, the interaction with the nonpolar stationary phase is relatively strong [31, 52]. HFIP covers the C18 stationary phase with a fluorous layer [52], shifting the stationary phase properties nearer to a fluorinated stationary phase. Acting as a weak ion-pairing agent, HFIP allows the alternative interaction with structurally similar FQs [52]. Using HFIP as an eluent additive decreases the retention of SAs more than FQs. At pH 9.0, the FQs exist mostly in the zwitterionic forms. Therefore, the retention pattern of FQs is influenced both by the fluorous layer of the stationary phase and by the acid-base equilibrium. At the same time, the SAs pKa values are much lower than 9 and the mobile phase pH does not affect the retention changes strongly.

In order to attain higher selectivity for FQs, the separation of five antimicrobials was studied in the alkyl perfluorinated

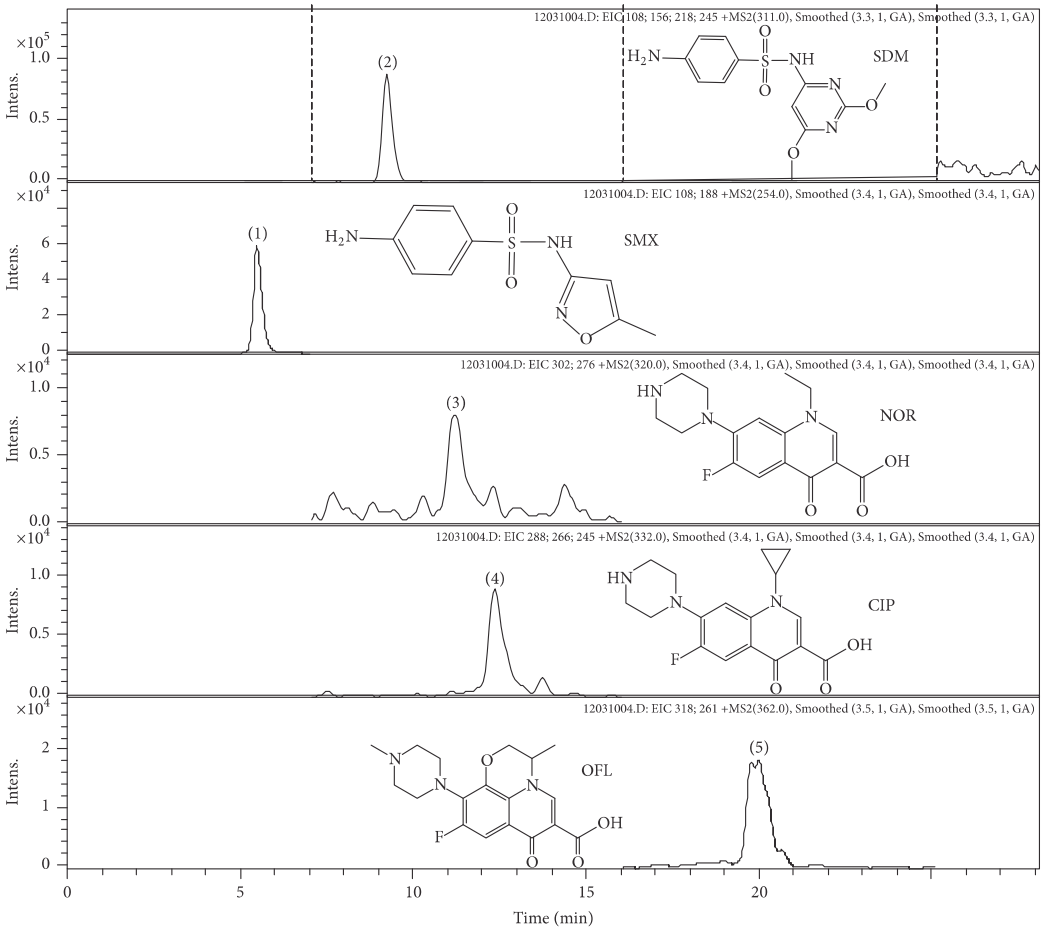

Figure 1: The chromatographic separation of antibiotic residues in potato tuber. Antimicrobials spiked in the LoQ level ((1) SMX 32.7 ng/g; (2) SDM 17.8 ng/g; (3) NOR 40.0 ng/g; (4) CIP 27.2 ng/g; (5) OFL 5.0 ng/g). Eluent: 5 mM HFIP (pH 9) and MeOH. Analytical column: Waters XBridge C18 column (150 mm × 3 mm, 3.5 μm).

stationary phase Epic FO-LB C8. However, the SMX and SDM showed well-expressed peak shapes and had different retention; the FQs peaks were wide and shallow, having similar retention in the perfluorinated stationary phase (Figure 2). Fluorinated analytes retention on the fluorinated stationary phase is mainly influenced by the number of fluorine atoms in the analyte molecule [53]. The number of fluorine atoms in the three FQs studied is one and the structures of the molecules are similar. Therefore, the retention of the analytes on the fluorinated stationary phase is also similar. On the other hand, the ESI signal of the analytes should be enhanced under acidic conditions. The MS chromatogram of the FQs and SAs separation had a high noise level in the extracted ion chromatograms for FQs; the peaks were broad and partly overlapping (Figure 2). Neither better separation nor enhanced signal was obtained by optimization of the elution gradient or the buffer composition or pH.

3.4. Method Validation. The described method was validated for the simultaneous determination of CIP, NOR, OFL, SDM, and SMX in plants. For calibration, antimicrobials and standard solutions were prepared in 10% methanol and water. The calibration graphs with peak area versus concentration were composed in concentration range 5–10,000 ng/g and were linear with $r^2 > 0.9998$. Extraction recovery was calculated from standard addition experiments. Extraction recoveries for all detected pharmaceuticals in all matrices varied from 54 to 98%; the average recoveries are shown in Figure 3. Method validation was performed in the matrix which showed the lowest recovery: carrot roots in loamy

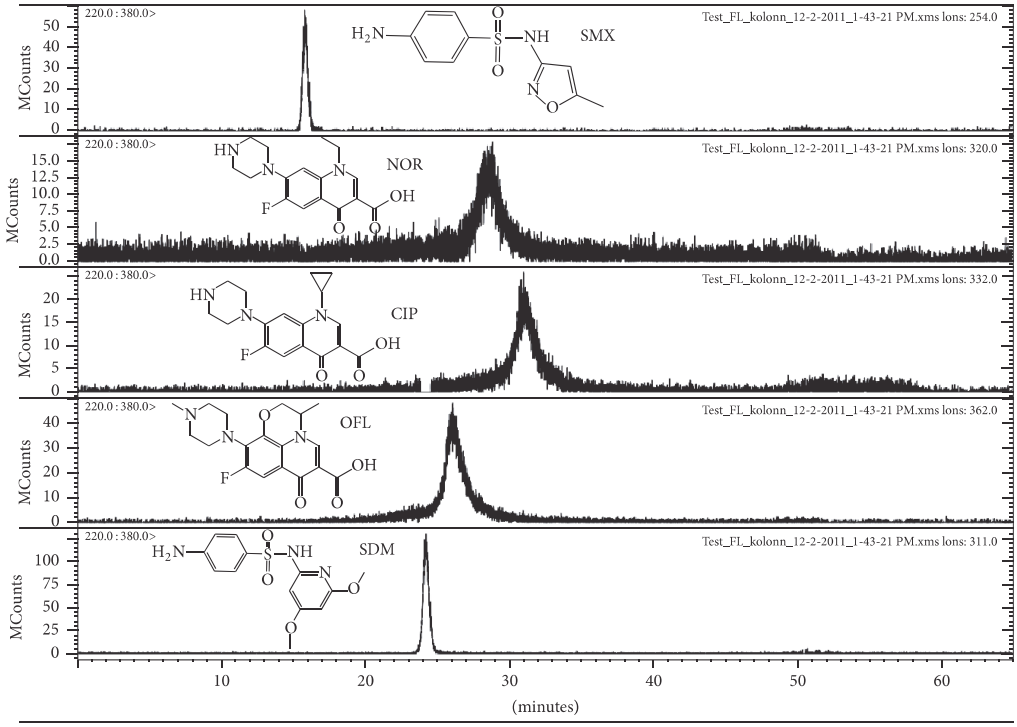

Figure 2: MS chromatogram for the standard solution of FQs and SAs (10 μg/g). Eluent: (ammonium acetate, formic acid pH 2.8) and MeOH. Used analytical column: Epic FO-LB C8 column (150 mm × 3 mm, 3.5 μm).

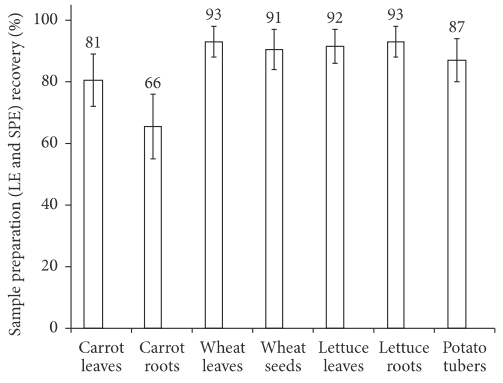

Figure 3: Average sample preparation (LE and SPE) recoveries (n = 2) of 5 antimicrobials (CIP, ciprofloxacin; NOR, norfloxacin; OFL, ofloxacin; SDM, sulfadimethoxine; SMX, sulfamethoxazole) from different parts of food plants grown in loamy soil using LE and SPE. Error bars show the recovery ranges.

soil (recovery ranges 54–78%, average recovery 66%). A postextraction spike in three different concentrations over the calibration range (low, 5 ng/g; medium, 250 ng/g; and high, 5,000 ng/g) to the different plants did not show a significant matrix interference. The process efficiency was primarily influenced by the extraction recovery in the two steps (LLE and SPE) of the sample preparation.

The average recoveries of antimicrobials from carrot roots, as shown in Figure 4, were 73% (CIP), 69% (NOR), 76% (OFL), 55% (SDM), and 70% (SMX). Standard deviations for the recoveries were 1% (CIP), 2% (NOR), 2% (OFL), 1% (SDM), and 1% (SMX). The limits of quantifications (LoQs) were estimated as ten times the standard deviation from five replicate analyses of unspiked and spiked plant samples using HLB cartridges. LoQs were as follows: CIP 27.2; NOR 40.0; OFL 5.0; SDM 17.8; and SMX 32.7 ng/g. The relative standard deviations (RSD) were, respectively, 0.27, 0.40, 0.05, 0.18, and 0.32 percent.

4. Conclusion

Plant uptake of pharmaceutical residues, present (even in very small amounts) in soils fertilized with sewage sludge compost, is an obvious reality. As antimicrobials consumed in very small amounts with everyday food can initiate strains of resistant bacteria in human and animal organisms, the high sensitivity of their detection methodology is of utmost

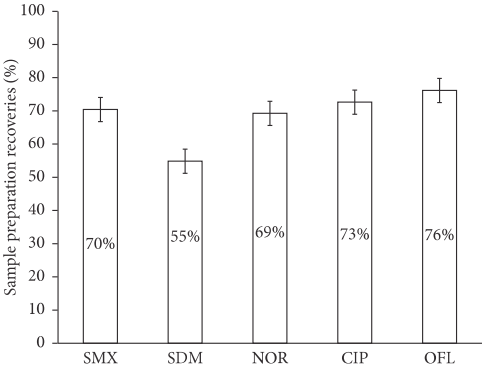

Figure 4: Average sample preparation recoveries for 5 antimicrobials from carrot roots using LE and SPE. Matrix: carrot roots. Error bars are 2 times standard deviation. CIP, ciprofloxacin; NOR, norfloxacin; OFL, ofloxacin; SDM, sulfadimethoxine; SMX, sulfamethoxazole.

importance. Improved separation of the groups of structurally similar antimicrobials, fluoroquinolones (FQs) and sulfonamides (SAs), and enhanced MS signal intensities were achieved as a result of this work, by using an eluent additive HFIP in regular C18 stationary phase. The developed and validated method described in the current paper has turned out to be an efficient tool for detecting the concentrations of antimicrobials in food plants fertilized with sewage sludge compost.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by Grant no. 7127 from the Estonian Science Foundation, by the Target Financing Project no. SF0180061s08 from the Ministry of Education and Science of Estonia, and by Estonian Environmental Investment Centre. The authors thank Ms. Karin Muoni and Mr. Andre Litvin for correcting this manuscript.

References

[1] E. Meyer, P. Gastmeier, M. Deja, and F. Schwab, "Antibiotic consumption and resistance: data from Europe and Germany," *International Journal of Medical Microbiology*, vol. 303, no. 6, pp. 388–395, 2013.

[2] K. J. Forsberg, A. Reyes, B. Wang, E. M. Selleck, M. O. A. Sommer, and G. Dantas, "The shared antibiotic resistome of soil bacteria and human pathogens," *Science*, vol. 337, no. 6098, pp. 1107–1111, 2012.

[3] K. Fent, A. A. Weston, and D. Caminada, "Ecotoxicology of human pharmaceuticals," *Aquatic Toxicology*, vol. 76, no. 2, pp. 122–159, 2006.

[4] S. Zühlke, U. Dünnbier, and T. Heberer, "Detection and identification of phenazone-type drugs and their microbial metabolites in ground and drinking water applying solid-phase extraction and gas chromatography with mass spectrometric detection," *Journal of Chromatography A*, vol. 1050, no. 2, pp. 201–209, 2004.

[5] K.-R. Kim, G. Owens, Y. S. Ok, W.-K. Park, D. B. Lee, and S.-I. Kwon, "Decline in extractable antibiotics in manure-based composts during composting," *Waste Management*, vol. 32, no. 1, pp. 110–116, 2012.

[6] C. E. Rodríguez-Rodríguez, E. Barón, P. Gago-Ferrero et al., "Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus Trametes versicolor in bioslurry reactor," *Journal of Hazardous Materials*, vol. 233-234, pp. 235–243, 2012.

[7] R. Reichel, I. Rosendahl, E. T. H. M. Peeters et al., "Effects of slurry from sulfadiazine-(SDZ) and difloxacin-(DIF) medicated pigs on the structural diversity of microorganisms in bulk and rhizosphere soil," *Soil Biology and Biochemistry*, vol. 62, pp. 82–91, 2013.

[8] E. Haiba, L. Nei, M. Ivask et al., "Sewage sludge composting and fate of pharmaceutical residues—recent studies in Estonia," *Agronomy Research*, vol. 14, pp. 1583–1600, 2016.

[9] O. Borgman and B. Chefetz, "Combined effects of biosolids application and irrigation with reclaimed wastewater on transport of pharmaceutical compounds in arable soils," *Water Research*, vol. 47, no. 10, pp. 3431–3443, 2013.

[10] D. Ašperger, V. Tišler, M. Zrnčić et al., "HPLC-DAD-FLD determination of veterinary pharmaceuticals in pharmaceutical industry wastewater with precolumn derivatization using fluorescamine," *Chromatographia*, vol. 77, no. 15, pp. 1059–1066, 2014.

[11] M. Motoyama, S. Nakagawa, R. Tanoue, Y. Sato, K. Nomiyama, and R. Shinohara, "Residues of pharmaceutical products in recycled organic manure produced from sewage sludge and solid waste from livestock and relationship to their fermentation level," *Chemosphere*, vol. 84, no. 4, pp. 432–438, 2011.

[12] A. Gonzalez-Martinez, A. Rodriguez-Sanchez, M. V. Martinez-Toledo et al., "Effect of ciprofloxacin antibiotic on the partial-nitritation process and bacterial community structure of a submerged biofilter," *Science of the Total Environment*, vol. 476-477, pp. 276–287, 2014.

[13] C. E. Rodríguez-Rodríguez, A. Jelić, M. Llorca et al., "Solid-phase treatment with the fungus Trametes versicolor substantially reduces pharmaceutical concentrations and toxicity from sewage sludge," *Bioresource Technology*, vol. 102, no. 10, pp. 5602–5608, 2011.

[14] T. Vasskog, O. Bergersen, T. Anderssen, E. Jensen, and T. Eggen, "Depletion of selective serotonin reuptake inhibitors during sewage sludge composting," *Waste Management*, vol. 29, no. 11, pp. 2808–2815, 2009.

[15] W. Peysson and E. Vulliet, "Determination of 136 pharmaceuticals and hormones in sewage sludge using quick, easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography-time-of-flight-mass spectrometry," *Journal of Chromatography A*, vol. 1290, pp. 46–61, 2013.

[16] M. Lillenberg, S. Yurchenko, K. Kipper et al., "Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting," *International Journal of Environmental Science and Technology*, vol. 7, no. 2, pp. 307–312, 2010.

[17] K. Kipper, K. Herodes, M. Lillenberg, L. Nei, E. Haiba, and S. V. Litvin, "Plant uptake of some pharmaceuticals commonly

present in sewage sludge compost," in *Proceedings of the 2nd International Conference on Chemical, Biological and Environmental Engineering (ICBEE '10)*, pp. 261–264, Cairo, Egypt, November 2010.

[18] L. Nei, E. Haiba, S. Kutti, M. Lillenberg, K. Kipper, and K. Herodes, "Sewage sludge compost, microbial activity and pharmaceuticals," *AWERProcedia Advances in Applied Sciences*, vol. 3, pp. 30–37, 2014.

[19] A. B. A. Boxall, P. Johnson, E. J. Smith, C. J. Sinclair, E. Stutt, and L. S. Levy, "Uptake of veterinary medicines from soils into plants," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 6, pp. 2288–2297, 2006.

[20] J. Qiting and Z. Xiheng, "Combination process of anaerobic digestion and ozonization technology for treating wastewater from antibiotics production," *Water Treat*, vol. 3, pp. 285–291, 1988.

[21] M. Ravina, L. Campanella, and J. Kiwi, "Accelerated mineralization of the drug Diclofenac via Fenton reactions in a concentric photo-reactor," *Water Research*, vol. 36, no. 14, pp. 3553–3560, 2002.

[22] H. F. Schröder, "Mass spectrometric monitoring of the degradation and elimination efficiency for hardly eliminable and hardly biodegradable polar compounds by membrane bioreactors," *Water Science and Technology*, vol. 46, no. 3, pp. 57–64, 2002.

[23] T. A. Ternes, J. Stüber, N. Herrmann et al., "Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater?" *Water Research*, vol. 37, no. 8, pp. 1976–1982, 2003.

[24] C. Lee, C. Schmidt, J. Yoon, and U. Von Gunten, "Oxidation of N-nitrosodimethylamine (NDMA) precursors with ozone and chlorine dioxide: kinetics and effect on NDMA formation potential," *Environmental Science and Technology*, vol. 41, no. 6, pp. 2056–2063, 2007.

[25] C. Trautwein, K. Kümmerer, and J. W. Metzger, "Aerobic biodegradability of the calcium channel antagonist verapamil and identification of the dead-end metabolite 2-(3,4-dimethoxyphenyl)-2isopropyl-5-(methylamino)pentane nitrile," *Chemosphere*, vol. 72, pp. 442–450, 2008.

[26] F. Méndez-Arriaga, S. Esplugas, and J. Giménez, "Photocatalytic degradation of non-steroidal anti-inflammatory drugs with TiO_2 and simulated solar irradiation," *Water Research*, vol. 42, no. 3, pp. 585–594, 2008.

[27] EMEA/MRL/026/95, Committee for Veterinary Medicinal Products, Sulphonamides (2), Summary report, 1995, http://www.ema.europa.eu/ema/index.jsp?curl=pages/includes/document/document_detail.jsp?webContentId=WC500015931&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058009a3dc.

[28] EMEA/MRL/820/02 - FINAL, Committee for Veterinary Medicinal Products, Enrofloxacin (extension to all food producing species), Summary report (5), 2002, http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014151.pdf.

[29] E. M. Golet, A. Strehler, A. C. Alder, and W. Giger, "Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followed by solid-phase extraction," *Analytical Chemistry*, vol. 74, no. 21, pp. 5455–5462, 2002.

[30] J. L. Stevens, G. L. Northcott, G. A. Stern, G. T. Tomy, and K. C. Jones, "PAHs, PCBs, PCNs, organochlorine pesticides, synthetic musks, and polychlorinated n-alkanes in U.K. sewage sludge: Survey results and implications," *Environmental Science and Technology*, vol. 37, no. 3, pp. 462–467, 2003.

[31] K. Kipper, K. Herodes, I. Leito, and L. Nei, "Two fluoroalcohols as components of basic buffers for liquid chromatography electrospray ionization mass spectrometric determination of antibiotic residues," *Analyst*, vol. 136, no. 21, pp. 4587–4594, 2011.

[32] A. Göbel, C. S. McArdell, M. J.-F. Suter, and W. Giger, "Trace determination of macrolide and sulfonamide antimicrobials, a human sulfonamide metabolite, and trimethoprim in wastewater using liquid chromatography coupled to electrospray tandem mass spectrometry," *Analytical Chemistry*, vol. 76, no. 16, pp. 4756–4764, 2004.

[33] A. Göbel, A. Thomsen, C. S. McArdell et al., "Extraction and determination of sulfonamides, macrolides, and trimethoprim in sewage sludge," *Journal of Chromatography A*, vol. 1085, no. 2, pp. 179–189, 2005.

[34] C. A. Kinney, E. T. Furlong, S. D. Zaugg et al., "Survey of organic wastewater contaminants in biosolids destined for land application," *Environmental Science and Technology*, vol. 40, no. 23, pp. 7207–7215, 2006.

[35] T. L. Jones-Lepp and R. Stevens, "Pharmaceuticals and personal care products in biosolids/sewage sludge: the interface between analytical chemistry and regulation," *Analytical and Bioanalytical Chemistry*, vol. 387, no. 4, pp. 1173–1183, 2007.

[36] J. Heidler, A. Sapkota, and R. U. Halden, "Partitioning, persistence, and accumulation in digested sludge of the topical antiseptic triclocarban during wastewater treatment," *Environmental Science and Technology*, vol. 40, no. 11, pp. 3634–3639, 2006.

[37] A. Kaleta, M. Ferdig, and W. Buchberger, "Semiquantitative determination of residues of amphetamine in sewage sludge samples," *Journal of Separation Science*, vol. 29, no. 11, pp. 1662–1666, 2006.

[38] T. Okuda, N. Yamashita, H. Tanaka, H. Matsukawa, and K. Tanabe, "Development of extraction method of pharmaceuticals and their occurrences found in Japanese wastewater treatment plants," *Environment International*, vol. 35, no. 5, pp. 815–820, 2009.

[39] L. Migliore, G. Brambilla, S. Cozzolino, and L. Gaudio, "Effect on plants of sulphadimethoxine used in intensive farming (Panicum miliaceum, Pisum sativum and Zea mays)," *Agriculture, Ecosystems and Environment*, vol. 52, no. 2-3, pp. 103–110, 1995.

[40] L. Migliore, G. Brambilla, P. Casoria, C. Civitareale, S. Cozzolino, and L. Gaudio, "Effect of sulphadimethoxine contamination on barley (Hordeum distichum L., Poaceae, Liliopsida)," *Agriculture, Ecosystems and Environment*, vol. 60, no. 2-3, pp. 121–128, 1996.

[41] G. Brambilla, P. Casoria, C. Civitareale, S. Cozzolino, L. Gaudio, and L. Migliore, "Sulphadimetoxine as environmental tracer to evaluate phytotoxity in crop," in *Proceedings of the Conference on Residues of Veterinary Drugs in Food*, pp. 292–295, Veldhoven, Netherlands, 1996.

[42] P. K. Jjemba, "The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land: a review," *Agriculture, Ecosystems and Environment*, vol. 93, no. 1-3, pp. 267–278, 2002.

[43] L. Migliore, S. Cozzolino, and M. Fiori, "Phytotoxicity to and uptake of enrofloxacin in crop plants," *Chemosphere*, vol. 52, no. 7, pp. 1233–1244, 2003.

[44] M. Lillenberg, M. Roasto, and T. Püssa, "Drug residues in environment. Estimation of fluoroquinolones in soil and food plants," *Journal of Agricultural Science*, vol. 14, pp. 13–26, 2003.

[45] H. Dolliver, K. Kumar, and S. Gupta, "Sulfamethazine uptake by plants from manure-amended soil," *Journal of Environmental Quality*, vol. 36, no. 4, pp. 1224–1230, 2007.

[46] L. Michelini, R. Reichel, W. Werner, R. Ghisi, and S. Thiele-Bruhn, "Sulfadiazine uptake and effects on Salix fragilis L. and Zea mays L. plants," *Water, Air, and Soil Pollution*, vol. 223, no. 8, pp. 5243–5257, 2012.

[47] A. Thomsen, A. Göbel, Mc. Ardell C et al., "Determination of sulfonamide and macrolide antibiotics in sewage sludge using accelerated solvent extraction and LC-MS/MS," in *Proceeddings of the Poseidon Symposium*, Braunschweig, Germany, 2003.

[48] M. Lillenberg, S. Yurchenko, K. Kipper et al., "Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry," *Journal of Chromatography A*, vol. 1216, no. 32, pp. 5949–5954, 2009.

[49] M. Lillenberg, *Residues of some pharmaceuticals in sewage sludge in Estonia, their stability in the environment and accumulation into food plants via fertilizing: PhD thesis in environmental protection [Ph.D. thesis]*, Estonian University of Life Sciences, Tartu, Estonia, 2011.

[50] H. Holcombe, Ed., *EURACHEM Guide: The Fitness for Purpose of Analytical Methods—A Laboratory Guide to Method Validation and Related Topics*, LGC, Teddington, UK, 1998.

[51] M. E. Lindsey, M. Meyer, and E. M. Thurman, "Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry," *Analytical Chemistry*, vol. 73, no. 19, pp. 4640–4646, 2001.

[52] K. Kipper, K. Herodes, and I. Leito, "Fluoroalcohols as novel buffer components for basic buffer solutions for liquid chromatography electrospray ionization mass spectrometry: retention mechanisms," *Journal of Chromatography A*, vol. 1218, no. 45, pp. 8175–8180, 2011.

[53] J. A. Gladysz, D. P. Curran, and I. T. Horvath, *Handbook of Fluorous Chemisry*, Wiley, New York, NY, USA, 2004.

PAPER III

Haiba, E., Nei, L. (2017). Sewage sludge composting and pharmaceuticals. *European Scientific Journal*, 114−121.

Sewage Sludge Composting and Pharmaceuticals

Egge Haiba, MSc
Lembit Nei, PhD
Tartu College, Tallinn University of Technology, Estonia

Abstract

Drug residues end up in the environment when sewage sludge or its compost is used as a fertilizer and they cause adverse effects there. Both, the producers and consumers seem to believe that drug residues decompose during sewage sludge treatment or in soil and do not affect the environment or humans. The acceptable level of drugs in different compartments of the environment is still disputable.

Keywords: Pharmaceuticals, Sewage sludge, Compost

Pharmaceuticals in the environment

Intensive use of pharmaceuticals in modern medicine and agriculture is the main reason for global environment pollution by these contaminants (Radović et al., 2016). Widespread occurrence of pharmaceuticals in the environment is well established (Daughton & Ruhoy, 2009). It has now been almost two decades since the defining papers by Halling-Sørensen (1998) and Daughton & Ternes (1999) identified pharmaceuticals in the environment as an important phenomenon. Medical substances have been measured in the effluent of medical care units, sewage and the effluent of sewage treatment plants, in surface water, ground water, and in drinking water (Heberer, 2002). Uncontrolled discharging of these organic compounds into the environmental media has led to their accumulation into soil and sediments (Radović et al., 2016).

Some pharmaceuticals are extremely persistent and introduced to the environment in very high quantities and perhaps have already gained ubiquity worldwide, others could act as if they were persistent, simply because their continual infusion into the aquatic environment serves to sustain perpetual life-cycle exposures for aquatic organisms (Daughton & Ternes, 1999). When drugs are detected in the environment, their concentrations are generally in the ng/L-µg/L (ppt-ppb) range (Moldovan et al., 2009). Even though individual concentrations of any drug might be low, the combined concentrations from drugs sharing a common mechanism of

action could be substantial (Daughton & Ternes, 1999). In 2013 Hughes et al. have published a global-scale analysis of the presence of 203 pharmaceuticals across 41 countries and showed that contamination is extensive due to widespread consumption and subsequent disposal to rivers. According to this overview, painkillers were globally the most frequently detected compounds accounting for 31% of records with a median concentration of 230 ng/L followed by antibiotics (21%, 8128 ng/ L).

There are increasing concerns about the undesired impacts that may result from continuous contamination of the environment with pharmaceutically-active substances (Barbosa et al., 2016 and Verlicchi & Zambello, 2016). One of the possible fates of pharmaceuticals is to accumulate in organisms. Bioaccumulation may have different effects from increased internal loads in a given organism potentially reaching toxic concentrations to biomagnification through up-concentration along a food chain (Straub, 2015).

Antibiotics present in soil contaminated with pharmaceutical residues may be taken up by plants from arable land or pasture, and thus involuntarily end up in human or animal food, destroy soil microorganisms or develop drug resistance. Genes determining drug resistance can be transferred from harmless soil microbes to pathogenic microbes (Davies, 1994). It is assumed that using sewage sludge or manure containing drug residues for fertilizing is one of the main reasons of increasing drug resistance (Knapp et al., 2010).

Since 1940 the production and use of antibacterial drugs has multiplied while the antibiotic resistance of bacteria has also increased noticeably. It has been shown that the occurrence of tetracycline resistant gene among soil bacteria increased 15 times between 1970–2008 in the Netherlands, which was caused by using sewage sludge or compost as a fertilizer (Knapp et al., 2010). Probably, the long-term influence of antibiotics on soil microbes has brought about the same effect also in other countries.

Pharmaceuticals in sewage sludge

The environmental presence of pharmaceuticals is attributed primarily to raw or treated sewage (for human drugs) and to manure and lagoons (for veterinary drugs); additional, less obvious sources also exist, which sometimes can play important localized roles (Daughton, 2007). The major route by which pharmaceuticals enter sewage is commonly accepted to be via urine and feces, with each contributing different relative amounts depending on the pharmacokinetics and structure of the individual compound (Winkler et al., 2008). Urban wastewater seems to be the dominant emission pathway for pharmaceuticals globally, although emissions from industrial production, hospitals, agriculture, and aquaculture are important locally

(Beek et al., 2016). Sewage sludge is an inevitable by-product of wastewater treatment. In Estonia about 360,000 − 500,000 tons of it is created annually. Sewage sludge, which is difficult to market, piles up at wastewater treatment plants. Many pollutants are not efficiently removed during sewage and sewage sludge treatment (Martín et al., 2015).

In Lillenberg et al. (2010) the reported highest concentrations (μg/kg) of the antimicrobials norfloxacin (NOR), ciprofloxacin (CIP), ofloxacin (OFL), sulfamethoxazole (SMX) and sulfadimethoxine (SDM) were in sewage sludge as follows: NOR – 162; CIP – 426; OFL – 39; SMX – 6; SDM – 20. In the study carried out by Motoyama et al. (2011), the highest concentrations of the studied pharmaceuticals in sewage sludge were: CIP – 130; SMX – 8; SDM – 3; carbamazepine CBZ – 46. In Martín et al. (2015) the relevant values were: NOR – 258; SMX – 20; OFL – 432; CBZ – 106; and in our recent study (unpublished results): CBZ – 66; diclofenac – 92; triclosan – 1800 (all in μg/kg).

For the prevention of the development of microbial resistance of humans and animals the concentration of antimicrobials in agricultural soil must be clearly under 0.1μg/kg (Lillenberg, 2011). The limited selection of results given above clearly shows that raw sewage sludge in not suitable for improving the quality of agricultural soils.

Fate of pharmaceuticals during sewage sludge composting

Sewage sludge may be regarded as hazardous waste but it can also be used as a fertilizer. Its safety with respect to pharmaceutical residues must be assessed before use (Kipper et al., 2011). Antibiotics are present in Estonian sewage sludge (as elsewhere) and their content may exceed the relevant trigger values for manure (Lillenberg et al., 2011). According to Nayak & Kalamdhad (2015) composting is one of the sustainable practices to convert sewage sludge into useful agricultural product because it is rich in organic matter, micro- and macronutrients, which are essential for plants growth and soil fauna to live. Alternatively, it has been stated that sewage compost cannot be used for agricultural purposes: it may contain an excess amount of chemical contaminants that can be assimilated by food crops (Lillenberg et al., 2010). However, sewage compost is rich in minerals, enabling long-lasting supply for the fast growth of plants (Järvis et al., 2016).

Since the 1960s, Estonia has been the major oil shale producer and consumer in the world (Kalda et al., 2015). 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). Since mid–

1990s the national average soil P balance has been negative in Estonia due to a sharp decrease in fertilizer use and availability of manure. The national average soil P balance varied in 2004–2009 from -10 to -5 kg P/ha. Currently crop production in Estonia largely takes place at the expense of soil P resources (Astover & Rossner, 2013). One of the most efficient ways to eliminate these problems is an intelligent preparation of solid waste composts (Haiba et al., 2016).

Sawdust has been proven to be a good bulking agent for sludge composting (Banegas et al., 2007). In the study carried out by Qiu et al. (2012) the degradation of 4 sulfonamides using manure + sawdust or manure + rice straw was more effective than in the case of using manure alone (presumably due to the higher microorganism activities in the former). It has been shown by Kim et al., 2012, that sawdust could be a potential organic source able to initiate efficient composting, as exhibited by elevated composting temperatures, and consequently resulted in the reduction of residual concentrations of tetracyclines, sulfonamides and macrolides to reasonable levels in a relatively short composting period. Thus manure–based composts manufactured through the proper composting process can be acceptable for application to agricultural areas. However, application of livestock manure as raw manure and/or as liquid fertilizer after only a short storage period to stabilize the manure should be avoided as this may result in the potential release of veterinary antibiotics to the environment (Kim et al., 2012).

The degradation rate of pharmaceuticals in sewage sludge compost depends on the applied composting technology. The degradation of salinomycin was observed by Ramaswamyunder et al. (2015) under open and composting conditions. Composting with hay significantly reduced the concentration of salinomycin in the manure, making application of the post-compost manure safer for field application.

It has been shown, that the degradation of fluroquinolones (ciprofloxacin CIP, norfloxacin NOR and ofloxacin OFL) and sulfonamides (sulfadimethoxine SMX and sulfamethoxazole SDM) takes place during sewage sludge co-composting with sawdust, peat and straw (Haiba et al., 2013). Additions of sawdust clearly speeded up the decomposition of the studied pharmaceuticals, whereas the mixtures with peat and straw showed lower abilities to decompose pharmaceutical residues.

In compost mixtures with sawdust the concentrations of the studied pharmaceuticals decreased as much as 95% to 100% during 4-months composting period. The mixtures with straw and peat where less efficient in decomposing these pollutants: in the mixture with peat the degradation level for SMX was 83% and for SDM 76%; in the mixture with straw the degradation level for NOR was 79% and for OFL 74%. At the same time, the

concentrations of the other studied pharmaceuticals decreased more than 90% during the 4-month period.

The temperature profiles of the sewage sludge–sawdust mixture samples during composting are demonstrated in figure 1. Initially the temperature of the composting samples ranged from 20 to 38 ^{0}C (mesophilic stage), then rose to 42 ^{0}C in 8 days (start of thermophilic stage).

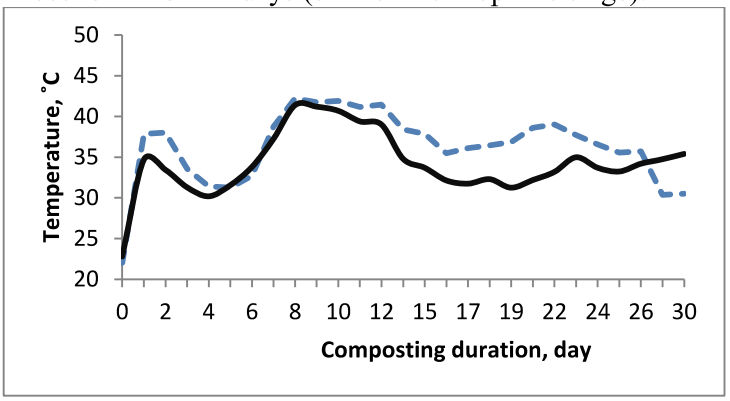

Figure 1. Temperature profiles during sewage sludge composting: sewage sludge mixed with sawdust - - - - E1 1:2 (v:v) and E2 1:3 (v:v)

In small volumes of sawdust-sludge samples, where the sample temperature remained unchanged, the degradation of pharmaceuticals was very slow. During 1-month period only 37% of the initial amount of SMX degraded (the lowest value); the highest level of degradation was apparent in the case of OFL - 82%. This clearly shows that composting may sufficiently speed up the degradation of pharmaceuticals originating from sewage sludge.

Although the results obtained in the case of composting sewage sludge with sawdust seem to be very promising, the experiments show very slow rate of carbamazepine degradation, not exceeding 20% during the 1-month period. According to this fact, the problems associated with the usage of sewage sludge compost as an agricultural fertilizer are far from reaching a final solution.

References:

Astover, A. & Rossner, H. 2013. Knowledge report: Phosphorus status of agricultural soils in Estonia. Baltic Forum for Innovative Technologies for Sustainable Manure Management, p 8.

Banegas, V., Moreno, J.L., Moreno, J.I., García, C., León, G. & Hernández, T. 2007. Composting anaerobic and aerobic sewage sludges using two proportions of sawdust. Waste Management 27, no. 10: 1317–1327.

Barbosa, M.O., Moreira, N.F.F., Riberio, A.R., Pereira, M.F.R. & Silva, A.M.T. 2016. Occurrence and removal of organic micropollutants: an

overview of the watch list of EU Decision 2015/495. Water Research 94: 257–279.
Beek, T. aus der, Weber, F.A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A. & Küster, A. 2016. Pharmaceuticals in the environment—Global occurrences and perspectives. Environmental Toxicology and Chemistry 35, no. 4: 823–835.
Daughton, C.G. 2007. Pharmaceuticals in the environment: Sources and their management. In Petrovic, M. & Barcelo, D., eds. Analysis, Fate and Removal of Pharmaceuticals in the Water Cycle, Vol 50. Wilson & Wilson's Comprehensive Analytical Chemistry Series. Elsevier Science, Amsterdam, The Netherlands: 1-58.
Daughton, C.G. & Ruhoy, I.S. 2009. Environmental footprint of pharmaceuticals: The significance of factors beyond direct excretion to sewers. Environmental Toxicology and Chemistry. 28, no. 12: 2495–2521.
Daughton, C.G. & Ternes, T.A. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environal Health Perspectives 107, suppl. 6: 907–938.
Davies, J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. Science 264, no. 5157: 375–382.
Ferrari, B., Mons, R., Vollat, B., Fraysse, B., Paxēaus, N., Giudice, R.Lo, Rollio, A. & Garric, J. 2004. Environmental risk assessment of six human pharmaceuticals: are the current risk assessment procedures sufficient for the protection of the aquatic environment. Environmental Toxicology and Chemistry 23, no. 5: 1344–1345.
Haiba, E., Nei, L., Ivask, M., Peda, J., Järvis, J., Lillenberg, M., Kipper, K. & Herodes, K. 2016. Sewage sludge composting and fate of pharmaceutical residues – recent studies in Estonia. Agronomy Research 14, no. 5: 1583-1600.
Haiba, E., Nei, L., Lillenberg, M., Kipper, K. & Herodes, K. 2013. Degradation of some pharmaceuticals during composting. AWERProcedia Advances in Applied Sciences 1: 857-862.
Halling-Sorensen, B., Nielsen, S.N., Lanzky, P.F., Ingerslev, F., Lutzhoft, H.C.H. & Jorgensen S.E. 1998. Occurrence, fate and effects of pharmaceutical substances in the environment − a review. Chemosphere 36, no. 2: 357–394.
Heberer, T. 2002. Occurrence, fate, and removal of pharmaceutical residus in the aquatic environment: a review of recent research data. Toxicology Letters 131, no. 1-2: 5–17.
Hughes, S.R., Kay, P., Brown, L.E. 2013. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environmental Science & Technology 47, no. 2: 661−677.

Järvis, J., Ivask, M., Nei, L., Kuu, A. & Luud, A. 2016. Effect of green waste compost application on afforestation success. Baltic Forestry 22, no. 1: 90-97.
Kalda, K., Ivask, M., Kutti, S., Kuu, A., Meriste, M., Nei, L., Peda, J. & Raukas, A. 2015. Soil invertebrates in semi-coke heaps of Estonian oil shale industry. Oil Shale 32, no. 1: 82–97.
Kim, K.-R., Owens, G., Ok, Y.S.; Park, W.-K., Lee, D.B. & Kwon, S.-I. 2012. Decline in extractable antibiotics in manure-based composts during composting. Waste Management 32, no.1: 110–116.
Kipper, K., Herodes, K., Leito, I. & Nei, L. 2011. Two fluoroalcohols as components of basic buffers for liquid chromatography electrospray ionization mass spectrometric determination of antibiotic residues. Analyst 136, no. 21: 4587-4594.
Knapp, C.W., Dolfing, J., Ehlert, P.A.I. & Graham, D.W. 2010. Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. – Environmental Science & Technology 44, no. 2: 580–587.
Lillenberg, M. 2011. Residues of some pharmaceuticals in sewage sludge in Estonia, their stability in the environment and accumulation into food plants via fertilizing: PhD thesis in environmental protection. Estonian University of Life Sciences.
Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Sepp, K., Lõhmus, R. & Nei, L. 2009. Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry. Journal of Chromatography A 1216, no. 32: 5949-5954.
Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Lõhmus, R., Ivask, M., Kuu, A., Kutti, S., Litvin, S.V. & Nei, L. 2010. Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting. International Journal of Environmental Science and Technology 7, no. 2: 307-312.
Martín, J., Santos, J.L., Aparicio, I. & Alonso, E. 2015. Pharmaceutically active compounds in sludge stabilization treatments: anaerobic and aerobic digestion, wastewater stabilization ponds and composting. Science of the Total Environment 503, special issue: 97–104.
Moldovan, Z., Chira, R. & Alder, A.C. 2009. Environmental exposure of pharmaceuticals and musk fragrances in the Somes River before and after upgrading the municipal wastewater treatment plant Cluj-Napoca, Romania. Environmental Science and Pollution Research 16, suppl. 1: 46-54.
Nayak, A.K. & Kalamdhad, A.S. 2015. Sewage sludge composting in a rotary drum reactor: stability and kinetic analysis. International Journal of Recycling of Organic Waste in Agriculture 4, no. 4: 249-259.

Motoyama, M., Nakagawa, S., Tanoue, R., Sato, Y., Nomiyamac, K. & Shinoharaa, R. 2011. Residues of pharmaceutical products in recycled organic manure produced from sewage sludge and solid waste from livestock and relationship to their fermentation level. Chemosphere 84, no. 4: 432-438.
Qiu, J.R., He, J.H., Liu, Q.Y., Guo, Z.Y., He, D.C., Wu, G.Y. & Xu, Z.C. 2012. Effects of conditioners on sulfonamides degradation during the aerobic composting of animal manures. Procedia Environmental Sciences 16: 17-24.
Radović, T.T., Grujić, S.D., Kovačević, S.R., Laušević, M.D. & Dmkić M.A. 2016. Sorption of selected pharmaceuticals and pesticides on different river sediments. Environmental Science and Pollution Research 23, no. 24: 25232–25244.
Ramaswamy, J., Prasher, S.O., Patel, R.M., Hussain, S.A. & Barrington ,S.F. 2010. The effect of composting on the degradation of a veterinary pharmaceutical. Bioresource Technology 101, no. 7: 2294–2299.
Straub, J. O. 2016. Aquatic environmental risk assessment for human use of the old antibiotic sulfamethoxazole in Europe. Environmental Toxicology and Chemistry 35, no. 4: 767–779.
Verlicchi, P. & Zambello, E. 2016. Predicted and measured concentrations of pharmaceuticals in hospital effluents. Examination of the strengths and weaknesses of the two approaches through the analysis of a case study. Science of the Total Environment 565: 82–94.
Winkler, M., Faika, D., Gulyas, H. & Otterpohl, R. 2008. A comparison of human pharmaceutical concentrations in raw municipal wastewater and yellowwater. Science of the Total Environment 399, no.1-3: 96-104.

PAPER IV

Haiba, E., Nei, L., Ivask, M., Peda, J., Järvis, J., Lillenberg, M., Kipper, K., Herodes, K. (2016). Sewage sludge composting and fate of pharmaceutical residues – recent studies in Estonia. *Agronomy Research*, 14 (5), 1583−1600.

Agronomy Research **14**(5), 1583–1600, 2016

Sewage sludge composting and fate of pharmaceutical residues – recent studies in Estonia

E. Haiba[1], L. Nei[1,*], M. Ivask[1], J. Peda[1], J. Järvis[1], M. Lillenberg[2], K. Kipper[3] and K. Herodes[3]

[1]Tartu College, Tallinn University of Technology, Puiestee 78, EE51008 Tartu, Estonia
[2]Estonian University of Life Sciences, Kreutzwaldi 58A, EE51014 Tartu, Estonia
[3]Institute of Chemistry, University of Tartu, Ravila 14A, EE51010 Tartu, Estonia
[*]Correspondence: lembit.nei@ttu.ee

Abstract. This review is to reflect the work addressed to the application of biosolids and especially sewage sludge as a resource in composting. A considerable drop in the use of P fertilisers can be followed since early 1990s. Due to this fact crop production in Estonia takes place at the expense of soil phosphorous (P) resources. One of the ways of increasing the fertility of agricultural lands is to use nutrient-rich sewage sludge. Unfortunately, this may cause several undesired consequences due to biological and chemical contaminants. The presence of some widely used pharmaceuticals, as ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFL), sulfadimethoxine (SDM) and sulfamethoxazole (SMX), was evident in sewage sludge of the two Estonian largest cities, Tartu and Tallinn. The concentrations of pharmaceuticals decreased after sewage sludge digestion and composting, but they were still present in detectable amounts. Sewage sludge co-composting experiments with sawdust, peat and straw showed the degradation of fluoroquinolones (FQ) and sulfonamides (SA). Additions of sawdust clearly speeded up this process, whereas the mixtures with peat and straw performed lower abilities to decompose pharmaceutical residues. Novel methodologies were developed and experiments conducted to study the potential accumulation of fluoroquinolones FQs and SAs by food plants. Due to the low adsorption of SAs on soil particles they are 'free' to migrate into plants. Different behaviour is characteristic to FQs as they are accumulated in sludge. Recent years have also shown progress in vermicomposting work and in using compost in afforestation.

Key words: composting technologies, fertilizers, pharmaceuticals, plant uptake, sewage sludge, vermicomposting.

INTRODUCTION

Land application of biosolids is generally considered to be the best option of disposal because it offers the possibility of recycling nutrients, provides organic material, improves soil properties, and enhances crop yields (White et al., 2011). Higher soil quality is generally associated with higher concentrations of soil organic matter and a plentiful supply of essential elements. Thus, the recycling of organic matter from anthropogenic residues to soil often benefits agricultural sustainability (White et al., 2013). However, this benefit has to be weighed against potential deleterious effects (White et al., 2011). Whilst recognising its significant value as a resource, recycling

sewage sludge to agricultural land requires a careful management to avoid potential negative impacts on the environment from chemical contaminants (Torri et al., 2012).

Organic residues recycling via composting appears to be an ancient activity. The practice of converting animal manure and other biodegradable wastes to compost is believed to have originated as early as agriculture (Fitzpatrick et al., 2005). The earliest known written reference to composting is found in clay tablets dated to the Akkadian empire, about 4,300 years ago (Rodale, 1960), but it is believed that the fertilizer value of aerobically degraded organic matter, which we now call compost, was recognized much earlier. There is evidence that the Romans, Greeks, and the Bani Israel knew about compost. The Bible and Talmud both contain numerous references to the use of rotted manure straw, and mention of compost occurs in 10th and 12th century Arab writings, in medieval Church texts, and in Renaissance literature (Smith et al., 2007).

A worldwide massive use of biosolids as soil conditioners and fertilizers arose in the early 1900s (Frank, 1998). Increasing urbanization and industrialization have resulted in a dramatic growth in the amount of wastes generated globally, particularly of sewage sludge as a byproduct from sewage treatment (White et al., 2011). Land application of treated sewage sludge and other biosolids improves soil fertility and has an important role in closing nutrient cycles (Torri et al., 2012). Among the macronutrients contained in sludge, phosphorus is an essential element for plant metabolism, often considered one of the most limiting nutrients for plant productivity (Shaheen et al., 2012).

A large variety of plant, animal and synthetic wastes can be gainfully composted at scales varying from a household bin to a large industry (Gajalakshmi & Abbasi, 2008). In the composting process, aerobic microorganisms use organic matter as a substrate (Gajalakshmi & Abbasi, 2008). The microorganisms decompose the substrate, breaking it down to more simple compounds (Epstein, 1997; Ipek et al., 2002). During composting, carbon- and nitrogen-containing compounds are transformed through successive activities of different microbes to more stable organic matter, which resembles humic substances (Pare et al., 1998). The rate and extent of these transformations depend on available substrates and the process variables used to control composting (Marche et al., 2003; Gajalakshmi & Abbasi, 2008).

Inventories of soil productive capacity indicate human-induced soil degradation on nearly 40% of the world's arable land (Doran & Zeiss, 2000); this warns us of the ecological collapse of the world's productive soils (Pankhurst et al., 1997). In Estonia the highly industrialised and centralised agricultural production system collapsed in the late 1980s and early 1990s. The area of arable land (crop fields and cultural grasslands) decreased from about one million ha in the early 1990s to less than 0.6 million ha by 2003 (Statistics Estonia, 2006; Iital et al., 2014). Also, a considerable drop in the use of N and P fertilisers took place in the early 1990s when it constituted only about 13% of the peak in 1987–1988. Based on the data from Statistics Estonia in 1994–2001 the average annual consumption of commercial fertilisers was only 85 kg ha^{-1} and in 2009–2011 it reached the level of 120 kg ha^{-1} (Statistics Estonia, 2012; Iital et al., 2014). Since mid–1990s the national average soil P balance has been negative in Estonia due to a sharp decrease in fertilizer use and availability of manure. The national average soil P balance varied in 2004–2009 from -10 to -5 kg P ha^{-1}. Currently crop production in Estonia largely takes place at the expense of soil P resources (Astover & Rossner, 2013).

One of the most efficient ways to eliminate this problem is an intelligent usage of solid waste composts.

This overview is to reflect recent research performed mainly in Estonia in the area of composting. These studies involved different aspects of sewage sludge composting and compost usage; vermicomposting of different waste materials; possible undesired consequences associated with the application of composts in agriculture.

NECESSITY FOR COMPOSTING AND RESOURCES (BACKGROUND)

The soil cover of Estonia is relatively varied due to the alternation of carbonate and humus-rich soils with acid soils which are relatively poor in nutrients and organic matter (Köster & Kõlli, 2013). The lack of nutrients is especially obvious in the case of peatlands which cover 22.3% (10,091 km^2) of Estonia's territory, so restricting the usage of these lands for agricultural purposes. The awareness of the composition and properties of soil cover and its relationship with plant cover in different land use conditions is the basis of ecologically proper and sustainable management of land and soil resource (Köster & Kõlli, 2013).

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.

Biosolids can be used in biofuel production (Raud et al., 2014), leading to the incineration of organic matter. Perceived as a green energy source, the combustion of biosolids has received renewed interest. Still, anaerobic digestion is generally a more effective method than incineration for energy recovery, and digested biosolids are suitable for further beneficial use through land application (Wang et al., 2008). The use of biosolids as a source of organic matter may improve the physical and chemical properties of agricultural soils resulting in an increase in crop yields (Torri et al., 2014). The major potential source for making compost in Estonia is sewage sludge. The yearly generation of sewage sludge by Estonian sewage treatment plants is 30,000 tonnes dw.

Semi-coke is the waste product of oil shale industry and presents the hazard to the environment, due to its phenol and PAH content. One of the main problems of oil shale industry is how to treat semi-coke effectively (Wang et al., 2009). In 1993−2003 the volume of semi-coke formed in Estonian shale oil enterprises varied within 0.6 and 1.4 million tonnes annually (Pae et al., 2005). It has been established that the compost made from semicoke and sewage sludge increases the yield of the crops (Varnik et al., 2006).

The average quantity of biodegradable waste generation in Estonia from grocery stores during 2004–2010 was 9 thousand tonnes $year^{-1}$. The results of SWOT analysis published by Blonskaja et al. in 2014 showed that composting process is the best solution for kitchen wastes. It has been demonstrated that one of the ecologically and environmentally friendly alternatives to traditional technologies in organic wastes management is vermicomposting, especially in kitchen wastes treatment (Ivask et al., 2013; Peda & Kutti, 2013; Haiba et al., 2014; Sinha et al., 2014).

SEWAGE SLUDGE COMPOSTING AND ENVIRONMENTAL CONCERNS

Unprecedented growth in urban population has resulted in the generation of huge quantities of wastewater worldwide (Singh & Agrawal, 2010). Wastewater treatment facilities are responsible for treating large volumes of domestic and industrial sewage containing human waste. The treatment goal is to produce effluents of high enough quality for discharge back into the environment. Sewage sludge is a byproduct of this process and necessitates proper disposal (Walters et al., 2010; Zuloaga et al., 2012). Safe disposal of sewage sludge is one of the major environmental concerns (Singh & Agrawal, 2010).

Historically, sewage sludge has been disposed of by incineration, landfilling or ocean disposal (Bridle & Skrypski-Mantele, 2000). Nowadays, the most widespread method for sewage sludge disposal has become agricultural application, since it is the most economical outlet for sludge compared to incineration and landfilling (Zuloaga et al., 2012; Li et al., 2013; Chen et al., 2014). The use of sewage sludge in agriculture is one of the major causes of environmental pollution (Nouri et al., 2008). Although, sewage sludge and its compost offers an opportunity to recycle plant nutrients and organic matter to soil for crop production stimulating biological activity (Rodríguez et al., 2012; Zuloaga et al., 2012; Li et al., 2013; Haiba et al., 2014), its usage as a fertilizer is limited due to a large number of toxic pollutants found in this matter (Lillenberg et al., 2010a; Lillenberg, 2011).

Composting is recognized as one of the most important recycling options for sewage sludge (Hara & Mino, 2008; Dorival-García et al., 2015). Since sewage sludge is mainly composted in Estonia and often re-used in agriculture as a fertilizer, several composting methods are applicable, but the selection of the method is dependent on the investment and operation cost, time required to reach compost stability and maturity, the availability of land, origin of raw materials and bulking agents (Ruggieri et al., 2008; Mollazadeh, 2014; Nei et al., 2014).

Several sludge composting experiences have been shared in Estonia (Kanal & Kuldkepp, 1993; Varnik et al., 2006; Kriipsalu et al., 2008; Kriipsalu & Nammari, 2010; Lillenberg et al., 2010a; Holm & Heinsoo, 2013; Kuusik et al., 2014; Menert et al., 2014). The most common sewage sludge composting methods are: static piles, aerated static piles, windrow and in-vessel systems (Yue et al., 2008). There are many factors that affect the composting process, such as the proportions of the mixture, temperature, rate of aeration, oxygen consumption rates, compost pile size, moisture content, pH and carbon-to-nitrogen ratio (Luo et al., 2008; Chen et al., 2014; Malinska et al., 2014; Nayak & Kalamdhad, 2014). Also, microorganisms play a key role in composting processes and nutrient turnover, and even slight changes in microbial activity and community composition due to antimicrobial agents may result in poor compost quality and prolonged time needed for compost stability (Nei et al., 2014). Respiration is a global measure of the total microbial activity that can provide a reliable, repeatable and scientifically sound assessment of microbial activity, respirometry (CO_2 evolution rate and/or O_2 uptake rate) has been widely used to evaluate microbial activity and composting efficiency (Liang et al., 2003; Barrena Gómez et al., 2006). The second widely used parameter for the evaluation of microbial activity is microbial biomass-C, measured by the substrate induced respiration based on Platen & Wirtz, 1999. Also, one of the methods of obtaining information about the dynamics of composting processes is

the bacterial-to-fungal ratio (Joergensen & Wichern, 2008). The microbial community may reflect the evolution and performance of the composting process thus acting as an indicator of compost maturity (Nei et al., 2014; Wang et al., 2015).

Since sewage sludge has high moisture content it cannot be composted alone – in order to absorb moisture it should be mixed with dry materials, which act as bulking agent thereby improving the aeration and the compost quality (Nayak & Kalamdhad, 2014; Zhou et al., 2014). Sludge and bulking agent proportions in compost influence the composting reaction rate and the final compost quality. Sludge can be mixed with different bulking agents, sources of carbon, such as peat, straw, wood chips, leaves, ash, peat, sawdust (Komilis et al., 2011; Cukjati et al., 2012; Maulini-Duran et al., 2013; Malinska et al., 2014).

A range of studies has shown that some pharmaceuticals and personal care products (PPCPs) are neither completely removed by sewage treatment, nor completely degraded in the environment (Redshaw et al., 2008; Lillenberg et al., 2009; Lillenberg et al., 2010a; Jelic et al., 2011; Rodríguez-Rodríguez et al., 2012; Borgman & Chefetz, 2013; Haiba et al., 2013b; Narumiya et al., 2013; Reichel et al., 2013). Although, their concentrations are much lower than the levels of traditionally known organic pollutants, the potential long-term effects of these compounds to humans, plants and animals cannot be ignored (Lillenberg et al., 2009; Nei et al., 2014; Van Doorslaer et al., 2014; Prosser & Sibley, 2015; Bártíková et al., 2016).

FATE OF PHARMACEUTICAL RESIDUES DURING SEWAGE SLUDGE COMPOSTING

Pharmaceuticals have been used for decades to prevent and treat human and animal diseases (Zhang et al., 2008; Li et al., 2014). Recently, there has been increasing concern about the effects of pharmaceuticals in aquatic and terrestrial ecosystems, as they can affect the efficiency of microbial-mediated processes (the regeneration of nutrients, carbon and nitrogen circulation and digestion of pollutants) in the environment (Girardi et al., 2011; Jelic et al., 2011; Bergersen et al., 2012; Martín et al., 2012; Chen et al., 2013; Li et al., 2014).

As a result of regular industrial, agricultural and household activities, a variety of compounds enter into the environment, of which only a small percentage are studied for their toxicological effects on humans and the environment (Peysson & Vulliet, 2013). Approximately 4,000 drug substance is used in Europe (human and veterinary), of which may have responsive impact to the environment (Rodríguez-Rodríguez et al., 2011). About 150 medical compounds are studied that have been found in the environment, but mostly in water samples (Rivera-Utrilla et al., 2013; Li et al., 2014). For example, the Estonian Statistics on Medicines data show that over the years the proportion of consumption of different drugs has increased, both over-the-counter as well as prescription drugs (State Agency of Medicines, 2011; 2013). There is no reliable information of how many people actually do or do not consume their drugs, how many medicines are not administered and how many different compounds are thrown into the sewage system or to the garbage. The increasing proportions of administered drugs and personal care products is alarming because of the compound releases to the environment are not controlled (Motoyama et al., 2011; Gonzalez-Martinez et al., 2014), which

involves a potential threat to the environment (Vasskog et al., 2009; Rodríguez-Rodríguez et al., 2011; Peysson & Vulliet, 2013).

A wide variety of pharmaceutically active compounds are present in wastewater effluents, surface waters, and ground waters (GWRC, 2008), and the sewage treatment plants are unable to remove all these substances. The removal rates of individual drugs during passage through a sewage treatment plant have varied from 12 to 90% (Stumpf et al., 1999; Butkovskyi et al., 2016). The fate of pharmaceuticals may be divided into three principal routes (Richardson & Bowron, 1985):

1. The substance is ultimately mineralized to carbon dioxide and water;
2. The substance is lipophilic and not readily degradable, so part of the substance will be retained in the sludge. These substances are able to contaminate soil if the sludge is dispersed onto fields;
3. The substance is metabolised to a more hydrophilic form of the parent lipophilic substance, but is still persistent and therefore will pass the sewage treatment plant, ends up in the receiving waters (rivers, seas) and may therefore affect the aquatic organisms, if the metabolites are biologically active.

Presence of different pharmaceuticals in sewage sludge is apparent, but there is still a lack of information concerning the fate of pharmaceutical residues in the environment (Kümmerer, 2008; Lillenberg, 2011). Pharmaceuticals are often not readily degradable (Richardson & Bowron, 1985; Gavalchin & Katz, 1994; Marengo et al., 1997; Halling-Sørensen et al., 2002; Hamscher et al., 2002; Carballa et al., 2004). Still, remarkable amounts of pharmaceuticals enter the soil via fertilizing with sewage sludge (Golet et al., 2002; Haiba et al., 2013a).

Medical substances have many necessary properties to bio-accumulate and provoke change in ecosystems (Kipper et al., 2010; Baran et al., 2011). No trigger values exist for drug residues in sewage sludge neither in Estonia (Decree of Estonian Minister of the Environment) nor in the European Union (EU Council Directive 86/278/EEC; Lillenberg et al., 2009). The most closely related act is the EU directive EMEA/CVMP/055 establishing trigger values for drug residues in manure (EMEA/CVMP/055/96). The content of drug residues should not exceed 100 μg kg^{-1} in manure and 10 μg kg^{-1} in the soil fertilized with manure. Montforts (2005) suggests that these figures should be remarkably lower. Soil organisms, microflora and plants are directly exposed to contaminants in sludge-amended soils.

The presence and content of some widely used pharmaceuticals was determined in sewage sludge and in its compost in the two Estonian largest cities, Tartu and Tallinn (Lillenberg, 2011). The sewage sludge in Tartu was treated by composting – mixing with tree bark (volume ratio 1:1). The methane fermentation and mixing with peat (volume ratio 1:0.75) were used in Tallinn. The samples were taken from anaerobically digested sludge (before mixing with peat) in Tallinn and from untreated sludge (before composting) in Tartu. The concentrations of most of the pharmaceuticals (ciprofloxacin-CIP, norfloxacin-NOR, ofloxacin-OFL, sulfadimethoxine-SDM and sulfamethoxazole-SMX) decreased significantly after sewage sludge digestion and compost processes, but many of them were still present in compost. The degradation of pharmaceutical residues was more efficient in Tallinn probably due to anaerobic sludge digestion (compost was made by mixing the treated sewage sludge with peat) compared to the results obtained in Tartu (raw sewage sludge was mixed with tree bark). The results of the relevant pilot studies are described in detail in Lillenberg et al. (2010a) and Lillenberg (2011).

Interestingly, SDM was present in most sludge and in some compost samples, although this antimicrobial was not marketed any more during the years of 2007 and 2008 in Estonia. It is possible that 'old' supplies were put to use or small amounts of this chemical were imported from other countries (Lillenberg et al., 2010a; Nei et al., 2010).

According to Lillenberg (2011) the highest concentrations of pharmaceuticals were found in Tallinn sewage sludge: CIP 1,520 μg kg^{-1} and NOR 580 μg kg^{-1} (dm). The highest detected concentration of CIP exceeded the trigger value for manure (100 μg kg^{-1}) over four times. The concentrations of OFL (134 μg kg^{-1}), SDM (73 μg kg^{-1}) and SMX (22 μg kg^{-1}) were lower (Table 1). The average contents of antibiotics were: CIP 737 μg kg^{-1}, NOR 279 μg kg^{-1}, OFL 80 μg kg^{-1}, SDM 2 μg kg^{-1} and SMX 18 μg kg^{-1} (dm). As a rule, the concentrations of pharmaceuticals in Tallinn sewage sludge from were relatively low. Still, in some cases the concentrations of CIP, NOR and OFL were over the trigger value (Table 1).

Table 1. The highest concentrations of pharmaceuticals detected from Tallinn sewage sludge, μg kg^{-1} (dm) (reproduced from Lillenberg, 2011)

Month	CIP	NOR	OFL	SDM	SMX
January	**1,520**	**580**	**134**	3	22
February	67	67	17	73	5
March	58	31	8	3	1
April	58	33	3	n.d.	2
May	**150**	**215**	7	0.4	n.d.
June	**206**	**163**	17	n.d.	4
July	39	37	4	n.d.	n.d.
August	11	26	5	n.d.	4
September	0.4	0.4	n.d.	n.d.	n.d.
November	42	16	9	3	3
December	53	85	37	4	7

CIP – ciprofloxacin; NOR – norfloxacin; OFL – ofloxacin; SDM – sulfadimethoxine; SMX – sulfamethoxazole; n.d. – not detected.

In Tartu, contrarily, the concentrations of CIP and NOR were in most cases over the trigger value, the high content of OFL was detected only in August, September and October (Lillenberg, 2011). The content of sulfonamides (SAs – SDM and SMX) was quite low in both cities, under the trigger value set for drug residues in manure (100 μg kg^{-1}) (Tables 1, 2). In Tartu at least one of SAs was present in every sludge sample (Table 2). The contents of SMX were in the range of 0.0–22 μg kg^{-1}, and SDM 0.00–73 μg kg^{-1} (dm) in Tallinn. In Tartu contents of SMX were between 0.0–11 μg kg^{-1}, and SDM 0.0–32 μg kg^{-1} (dm). The highest concentrations of antimicrobials in sewage sludge from Tartu were: NOR – 439 μg kg^{-1} and CIP – 442 μg kg^{-1} (dm). OFL was present in every sludge sample from Tartu and the highest concentration was 157 μg kg^{-1} (dm) (Table 2).

Table 2. The highest concentrations of pharmaceuticals determined from Tartu sewage sludge, μg kg^{-1} (dm) (reproduced from Lillenberg, 2011)

Month	CIP	NOR	OFL	SDM	SMX
January	**315**	82	86	8	6
February	**423**	**263**	68	32	7
March	89	60	26	0.4	1
May	**174**	**264**	22	1	n.d.
June	**265**	**264**	47	n.d.	16
July	67	**104**	19	n.d.	6
August	**442**	**439**	**111**	24	n.d.
September	**231**	**188**	**157**	22	9
October	**259**	**126**	**149**	4	n.d.
November	**134**	**105**	33	6	11
December	71	40	32	9	6

CIP – ciprofloxacin; NOR – norfloxacin; OFL – ofloxacin; SDM – sulfadimethoxine; SMX – sulfamethoxazole; n.d. – not detected.

The degradation of pharmaceuticals was more efficient in the case of composting in Tallinn. During 12 months composting period the concentrations of all the studied pharmaceuticals diminished for 99.9%, whereas in Tartu this indicator showed the value on average 90 ± 4%. The only exception was SDM, which 'disappeared' fully in both cases. In Tallinn the anaerobically digested sludge was mixed with peat and composted. In Tartu raw sewage sludge was mixed with tree bark (1:1) and settled in piles. The media vas mixed at least twice per month during eight-months period. It has been shown, that a higher decrease of pharmaceuticals is observed after anaerobic digestion than after aerobic digestion, which can be explained by a higher degradation under anaerobic conditions (Martin et al., 2015).

The degradation rate of pharmaceutical residues is dependent on the initial components of the compost. Fine sawdust appears to be an excellent sewage sludge amendment: from the agricultural point of view, sludge co-composted with particularly fine-textured sawdust is claimed to be an excellent compost material to be applied to soils (Ammari et al., 2012; Nei et al., 2015). Kim et al. (2012) have shown that sawdust is able to initiate efficient composting, leading to elevated composting temperatures, and consequently resulting in the reduction of residual concentrations of pharmaceuticals to reasonable levels in a relatively short composting period.

According to Haiba et al. (2013b), composting remarkably reduces the concentrations of these pharmaceuticals. In most experiments their concentrations decreased by 95% or more during 4 months of composting (Table 3). The best results were obtained when the sludge was mixed with sawdust. In the case of using straw or peat instead the decomposition rates were lower. Additions of sawdust clearly speeded up this process, whereas the mixtures with peat and straw performed lower abilities to decompose pharmaceutical residues. No clear evidence was received concerning the impact of oil shale amendments on the degradation speed of the studied pharmaceuticals. Many studies have shown that sawdust has been proven to be a good bulking agent for sewage sludge composting (Banegas et al., 2007; Zorpas & Loizidou, 2008; Haiba et al., 2013a & 2013 b). The decline of tetracycline and sulfonamide concentrations was highly dependent on the presence of sawdust while there was no influence of sawdust on tylosin decline (Kim et al., 2012).

Table 3. Degradation of pharmaceuticals in sewage sludge compost mixtures during 4-months composting period, %

Bulking agent (% from dry matter)	**SMX**	**SDM**	**NOR**	**CIP**	**OFL**
1. peat (50)	83	77	90	92	100
2. sawdust (33)	100	99	96	95	100
3. sawdust + oil shale ash (29+14)	100	96	82	94	99
4. sawdust + wood chips (total 43)	100	99	91	98	86
5. straw (50)	99	98	79	90	74

CIP – ciprofloxacin; NOR – norfloxacin; OFL – ofloxacin; SDM – sulfadimethoxine; SMX – sulfamethoxazole.

PHARMACEUTICALS AND PLANT UPTAKE

The significance of the route involving the uptake of several medicines from soil by plants in terms of risk to human health is evident (Lillenberg et al., 2010b; Prosser & Sibley, 2015; Wu et al., 2015). As the compost made from sewage sludge contains detectable amounts of pharmaceutical residues, experiments were conducted to study the significance of their uptake into plants from soil under 'real' conditions. Therefore, experiments were performed to investigate the potential accumulation of the studied pharmaceuticals – fluoroquinolones (FQs) and sulfonamides (SAs) – taken up by food plants (namely – carrot, potato, lettuce, wheat) from the soil fertilized with sewage sludge or its compost. The results of these experiments are shown in Lillenberg et al. (2010a; 2010b), Kipper et al. (2010) and Nei et al. (2010).

The uptake of pharmaceuticals by the studied food plants was noticeable. It has been shown that due to the low adsorption of SAs on soil particles they readily migrate into plants (Haiba et al., 2013a). Different behaviour is characteristic to FQs due to their sorption to sewage sludge and soil particles (Golet et al., 2003). Therefore, as a rule, the content of SAs in the plants was higher. The content of the studied pharmaceuticals was higher in plats cultivated in sandy soil (Lillenberg, 2011). In loamy soil the molecules of both SAs and FQs attach to clay particles reducing their uptake by plants. Fig. 1 is to illustrate the said. The amounts of FQs going into potato do not depend much on soil type. The application of sewage sludge compost as a fertilizer and the following uptake of pharmaceuticals by food plants may cause contamination of these plants (Haiba et al., 2013a).

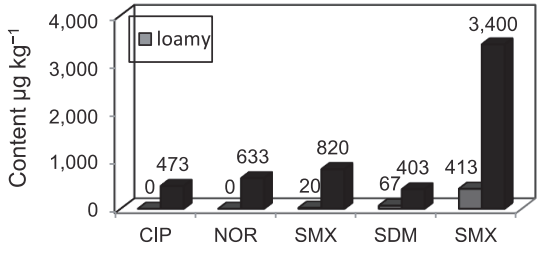

Figure 1. Average concentrations of pharmaceuticals in carrot roots grown in different soils at drug concentration of 10 mg kg^{-1}: CIP – ciprofloxacin; NOR – norfloxacin; OFL – ofloxacin; SDM – sulfadimethoxine; SMX – sulfamethoxazole.

Toxic compounds entering into the soil may affect microbial activity, plant growth and development and may have adverse effects on living organisms (Lillenberg et al., 2010b; Michelini et al., 2012; Haiba et al., 2013a; Nei et al., 2014). Further studies concerning the plant uptake of a wide spectrum of commonly used pharmaceuticals from soils fertilized with sewage sludge or its compost are needed to ensure food safety.

Lillenberg concludes in her PhD thesis (Lillenberg, 2011) that the residues of pharmaceuticals readily accumulate in several food plants. This phenomenon remarkably depends on the nature and concentration of a pharmaceutical and soil type. When using the sewage sludge compost as a fertilizer, it should be carefully tested for the safety. The content of pharmaceuticals in the compost made from sewage sludge may easily lead to the elevated concentrations in food plants if the compost is used as a fertilizer. Still, wheat grains had low or zero concentrations of the analysed pharmaceuticals. This confirmed the potential applicability of sewage sludge compost for fertilization of the crops of this type (Haiba et al., 2013a). Further work should be conducted to determine different types of pharmaceuticals and other organic pollutants by food plants (Lillenberg, 2011). It is evident that the development of novel sewage sludge treatment technologies are needed to solve environmental problems related to sewage sludge exploitation.

PUBLICATIONS AND THESES

Vermicomposting

Vermicomposting technology is a simple and environmentally friendly biological treatment of wastes. As a result of the work published in Ivask et al. (2013) and Haiba et al. (2014) the applicability and efficiency of using earthworms *Eisenia fetida* and *Dendrobaena veneta* in vermicomposting of sewage sludge and household organic residues in the countries with the climate comparable to Estonia was demonstrated.

Compost in afforestation

In Estonia the reforestation of depleted peat and sand mining areas is often complicated due to the unfavourable physical, chemical and biological properties of soils. The impact of artificial roots and soil amelioration with green waste compost in the afforestation of depleted peat fields and sand pits was studied. The results of this work is presented in Jarvis et al. (2012) and Jarvis et al. (2016). Added compost caused significantly improved height growth of the studies tree species seedlings, hence enhanced the growth conditions locally.

Development of novel methodologies for the determination of pharmaceutical residues

Novel approaches for the quantitative determination of traces of commonly used pharmaceuticals in sewage sludge and plants were developed (Lillenberg et al., 2009; Kipper et al., 2011; Kipper, 2012). The compounds were simultaneously extracted from sewage sludge by pressurized liquid extraction (PLE). A novel and effective method for PLE was developed. Solid-phase extraction was used for cleaning up the extracts.

Dissertations defended

PhD thesis: Karin Kipper, Fluoroalcohols as Components of LC-ESI-MS Eluents: Usage and Applications, 2012. A novel and efficient methodology for pharmaceutical analyses in complex matrices (e.g. blood plasma and environmental samples) was developed and tested.

PhD thesis: Merike Lillenberg, Residues of some pharmaceuticals in sewage sludge in Estonia, their stability in the environment and accumulation into food plants via fertilizing, 2011. The aim of the work was to study the presence of some widely used pharmaceuticals in Estonian sewage sludge and its compost and the uptake of these pharmaceuticals from fertilized soils by some food plants. As a result of this research the following was established:

1. Pharmaceuticals were present in sewage sludge and its compost from both Tallinn and Tartu and in several samples their concentrations exceeded the relevant trigger values for manure.
2. Degradation of pharmaceuticals took place as a result of composting.
3. The main reason of the decrease in pharmaceutical concentrations during composting was the applied sludge treatment technology.
4. The uptake of the studied pharmaceuticals by food plants was obvious. The application of sewage sludge compost as a fertilizer and the resulting uptake of pharmaceuticals by food plants may cause contamination of these plants.

CONCLUSIONS

Land application of composts is an important and efficient tool in the remediation of industrial landscapes and agricultural soils in Estonia. Still, due to the frequent presence of different undesired residues, composts made from sewage sludge need careful inspection before their use. The work should be continued by the development of novel and more efficient composting technologies, leading to intelligent solutions of environmental problems related to biowaste exploitation.

ACKNOWLEDGEMENTS. The authors would like to thank Environmental Investment Centre of Estonia for funding our research and Ms Karin Muoni for correcting this manuscript.

REFERENCES

Ammari, T.G., Al-Omari, Q. & Abbassi, B.E. 2012. Composting sewage sludge amended with different sawdust proportions and textures and organic waste of food industry – assessment of quality. *Environmental Technology* **33**, 1641–1649.

Astover, A. & Rossner, H. 2013, Knowledge report: Phosphorus status of agricultural soils in Estonia. *Baltic Forum for Innovative Technologies for Sustainable Manure Management*, p 8.

Banegas, V., Moreno, J.L., Moreno, J.I., Garcı´a, C., Leon, G. & Hernandez, T. 2007. Composting anaerobic and aerobic sewage sludges using two proportions of saadust. *Waste Management* **27**, 1317–1327.

Baran, W., Adamek, E., Ziemiańska, J. & Sobczak, A. 2011. Effects of the presence of sulfonamides in the environment and their influence on human healt. *Journal of Hazardous Materials* **196**, 1–15.

Barrena Gómez, R., Vázquez Lima, F. & Sánchez Ferrer, A. 2006. The use of respiration indices in the composting process: A review. *Waste Management & Research* **24**, 37–47.

Bártíková, H., Podlipná, R. & Skálová, L. 2016. Veterinary drugs in the environment and their toxicity to plants. *Chemosphere* **144**, 2290–2301.
Bergersen, O., Hanssen, K.Ø. & Vasskog, T. 2012. Anaerobic treatment of sewage sludge containing selective serotonin reuptake inhibitors. *Bioresource Technology* **117**, 325–32.
Blonskaja, V., Poldnurk, J. & Loigu, E. 2014. Utilisation options for biodegradable kitchen waste in Estonia. *SWOT analysis, Conference: 9th International Conference on Environmental Engineering (ICEE).* Vilnius, Lithuania, May 22–23.
Borgman, O. & Chefetz, B. 2013. Combined effects of biosolids application and irrigation with reclaimed wastewater on transport of pharmaceutical compounds in arable soils. *Water Research* **47**, 3431–3443.
Bridle, T. & Skrypski-Mantele, S. 2000. Assessment of sludge reuse options: a life cycle approach. *Water Science and Technology* **4**, 131–135.
Butkovskyi, A., Ni, G., Hernandez Leal, L., Rijnaarts, H.H.M. & Zeeman, G. 2016. Mitigation of micropollutants for black water application in agriculture via composting of anaerobic sludge. *Journal of Hazardous Materials* **303**, 41–47.
Carballa, M., Omil, F., Lema, J.M., Llompart, M., García-Jares, C., Rodríguez, I., Gómez, M. & Ternes, T. 2004. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Research* **38**, 2918–2926.
Chen, Y., Yu, F., Liang, S., Wang, Z., Liu, Z. & Xiong, Y. 2014. Utilization of solar energy in sewage sludge composting: Fertilizer effect and application. *Waste Management* **34**, 2014–2021.
Chen, Y., Yu, G., Cao, Q., Zhang, H., Lin, Q. & Hong, Y. 2013. Occurrence and environmental implications of pharmaceuticals in Chinese municipal sewage sludge. *Chemosphere* **93**, 1765–72.
Cukjati, N., Zupancic, G.D., Ros, M. & Grilc, V. 2012. Composting of anaerobic sludge: An economically feasible element of a sustainable sewage sludge management. *Journal of Environmental Management* **106**, 48–55.
Decree of Estonian Minister of the Environment No.78. 2004. State Gazette I, 9, 52, 2004, Estonia (in Estonian).
Doran, W.J. & Zeiss, M.R. 2000. Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology* **15**, 3–11.
Dorival-García, N., Labajo-Recio, C., Zafra-Gómez, A., Juárez-Jiménez, B. & Vílchez, J.L. 2015. Improved sample treatment for the determination of 17 strong sorbed quinolone antibiotics from compost by ultra high performance liquid chromatography tandem mass spectrometry. *Talanta* **138**, 247–257.
EMEA/CVMP/055/96. 1996. Committee for Veterinary Medicinal Products. Note for guidance: environmental risk assessment for veterinary medicinal products other than GMO-containing and immunological products. Veterinary Medicines Evaluation Unit, London, 1996.
Epstein, E. 1997. The science of composting. Technomic Publishing, Lancaster, PA.
EU Council Directive 86/278/EEC. 1986. On the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. European Community, Brussels, 12 June 1986.
Fitzpatrick, G.E., Worden, E.C. & Vendrame, W.A. 2005. Historical development of composting technology during the 20th century. *Hort Technology* **15**, 48–51.
Frank, R. 1998. The use of biosolids from wastewater treatment plants in agriculture. *Environmental Management* **9**, 165–169.
Gajalakshmi, S. & Abbasi, S.A. 2008. Solid Waste Management by Composting: State of the Art. *Critical Reviews in Environmental Science and Technology* **38**, 311–400.
Gavalchin, J. & Katz, S.E. 1994. The persistence of fecal born antibiotics in soil. *The Journal of AOAC International* **77**, 481–485.

Girardi, C., Greveb, J., Lamshöftc, M., Fetzerd, I., Miltnera, A., Schäffere, A. & Kästner, M. 2011. Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities. *Journal of Hazardous Materials* **198**, 22–30.

Golet, E.M., Strehler, A., Alder, A.C. & Giger, W. 2002. Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followed by solid-phase extraction. *Analytical Chemistry* **74**, 5455–5462.

Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C. & Giger, W. 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Environmental Science & Technology* **37**, 3243–3249.

Gonzalez-Martinez, A., Rodriguez-Sanchez, A., Martinez-Toledo, M.V., Garcia-Ruiz, M.J., Hontoria, E., Osorio-Robles, F. & Gonzalez-Lopez, J. 2014. Effect of ciprofloxacin antibiotic on the partial-nitritation process and bacterial community structure of a submerged biofilter. *Science of the Total Environment* **476–477**, 276–287.

GWRC – Global Water Research Coalition. 2008. Development of an International Priority List of Pharmaceuticals Relevant for the Water Cycle. Prepared by: Kiwa Water Research, CIRSEE and TZW, London, p.5.

Haiba, E., Lillenberg, M., Kipper, K., Astover, A., Herodes, K., Ivask, M., Kuu, A., Litvin, S.V. & Nei, L. 2013a. Fluoroquinolones and sulfonamides in sewage sludge compost and their uptake from soil into food plants. *African Journal of Agricultural Research* **8**, 3000−3006.

Haiba, E., Nei, L., Lillenberg, M., Kipper, K. & Herodes, K. 2013b. Degradation of some pharmaceuticals during sewage sludge composting. *Global Journal on Advances Pure and Applied Sciences*, *1st Global Conference on Environmental Studies* (CENVISU-2013), Belek, Antalya, Turkey, **1**, 857−862.

Haiba, E., Ivask, M., Olle, L., Peda, J., Kuu, A., Kutti, S. & Nei, L. 2014. Transformation of nutrients and organic matter in vermicomposting of sewage sludge and kitchen wastes. *Journal of Agricultural Science* **6**, 114–118.

Halling-Sørensen, B., Sengeløv, G. & Tjørnelund, J. 2002. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline resistant bacteria. *Archives of Environmental Contamination and Toxicology* **42**, 263–271.

Hamscher, G., Sczesny, S., Höper, H. & Nau, H. 2002. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. *Analytical Chemistry* **74**, 1509–1518.

Hara, K. & Mino, T. 2008. Environmental assessment of sewage sludge recycling options and treatment processes in Tokyo. *Waste Management* **28**, 2645–2652.

Holm, B. & Heinsoo, K. 2013. Municipal wastewater application to Short Rotation Coppice of willows – Treatment efficiency and clone response in Estonian case study. *Biomass & Bioenergy* **57**, 126−135.

Iital, A., Kloga, M., Pihlak, M., Pachel, K., Zahharov, A. & Loigu, E. 2014 Nitrogen content and trends in agricultural catchments in Estonia. *Agriculture Ecosystems & Environment* **198**, 44–53.

Ipek, U., Obek, E., Akca, L., Arslan, E.I., Hasar, H., Dogru, M. & Baykara, O. 2002. Determination of degradation of radioactivity and its kinetics in aerobic composting. *Bioresource Technology* **84**, 283–286

Ivask, M, Olle, L & Nei, L. 2013. Domestic organic waste treatment through vermitechnology. *Waste Management and Research* **31**, 878.

Jarvis, J., Ivask, M., Nei, L., Kuu, A. & Luud, A. 2016. Effect of Green Waste Compost Application on Afforestation Success. *Baltic Forestry* **22**, 90–97.

Jarvis, J., Ivask, M. & Nei, L. 2012 Artificial rope-wick roots improve the germination and establishment of tree species. *Seed Science and Technology* **40**, 433–436.

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sa´nchez, R., Ventura, F., Petrovic, M. & Barcelo, D. 2011. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. *Water Research* **45**, 1165–1176.

Joergensen, R.G. & Wichern, F. 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. *Soil Biology & Biochemistry* **40**, 2977–2991.

Kanal, A. & Kuldkepp, P. 1993. Direct and Residual Effect of Different Organic Fertilizers on Potato and Cereals. *Journal of Agronomy and Crop Science* **171**, 185–195.

Kim, K.R., Owens, G., Ok, Y.S., Park, W.K., Lee, D.B. & Kwon, S.I. 2012. Decline in extractable antibiotics in manure-based composts during composting. *Waste Management* **32**, 110–116.

Kipper, K., Herodes, K., Lillenberg, M., Nei, L., Haiba, E. & Litvin, S.V. 2010. Plant Uptake of some Pharmaceuticals Commonly Present in Sewage Sludge Compost. *Conference: 2nd International conference on Chemical, Biological and Environmental Engineering*, pp. 261–264.

Kipper, K., Herodes, K. & Leito, I. 2011. Two fluoroalcohols as components of basic buffers for liquid chromatography electrospray ionization mass spectrometric determination of antibiotic residues *Analyst* **136**, 4587–4594.

Kipper, K. 2012. PhD thesis, Fluoroalcohols as Components of LC-ESI-MS Eluents: Usage and Applications. Tartu University Press, p. 165.

Komilis, D., Evangelou, A. & Voudrias, E. 2011. Monitoring and optimizing the co-composting of dewatered sludge: A mixture experimental design approach. *Journal of Environmental Management* **92**, 2241–2249.

Kriipsalu, M., Marques, M., Hogland, W. & Nammari, D.R. 2008. Fate of polycyclic aromatic hydrocarbons during composting of oily sludge. *Environmental Technology* **29**, 43–53.

Kriipsalu, M. & Nammari, D. 2010. Monitoring of biopile composting of oily sludge. *Waste Management & Research* **28**, 395–40.

Kuusik, A., Pachel, K., Kuusik, A. & Loigu, E. 2014. Anaerobic co-digestion of sewage sludge with fish farming waste. *9th International Conference on Environmental Engineering (ICEE)*, Vilnius, Lithuania, 8 p.

Köster, T. & Kõlli, R. 2013. Soil–plant relationships dependent on land use. *Estonian Journal of Earth Sciences* **62**, 93–112.

Kümmerer, K. 2008. Pharmaceuticals in the Environment – a brief summary. In: Pharmaceuticals in the Environment. Sources, Fate, Effects and Risks. 3rd Edition. Kümmerer. K. (ed). Springer-Verlag, Berlin, Heidelberg, pp. 3–21.

Li, Y., Li, W., Liu, B., Wang, K., Su, C. & Wu, C. 2013. Ammonia emissions and biodegradation of organic carbon during sewage sludge composting with different ekstra carbon sources. *International Biodeterioration & Biodegradation* **85**, 624–630.

Li, Y., Zhu, G., Jern, Ng.W. & Tan, S.K. 2014. A review on removing pharmaceutical contaminants from waste water by constructed wetlands: Design, performance and mechanism. *Science of the Total Environment* **468–469**, 908–932.

Liang, C., Das, K.C. & McClendon, R.W. 2003. The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend. *Bioresource Technology* **86**, 131–137.

Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Sepp, K., Lõhmus, R. & Nei, L. 2009. Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry. *Journal of Chromatography A* **1216**, 5949–5954.

Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Lõhmus, R., Ivask, M., Kuu, A., Kutti, S., Litvin, S.V. & Nei, L. 2010a. Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting. *International Journal of Environmental Science & Technology* **7**, 307–312.

Lillenberg, M., Litvin, S.V., Nei, L., Roasto, M. & Sepp, K. 2010b. Enrofloxacin and Ciprofloxacin Uptake by Plants from Soil. *Agronomy Research* **8**, 807–814.

Lillenberg, M. 2011. PhD thesis, Residues of some pharmaceuticals in sewage sludge in Estonia, their stability in the environment and accumulation into food plants via fertilizing. (Estonian University of Life Sciences). p. 217, Tartu: Ecoprint.

Luo, W., Chen, T.B., Zheng, G.D., Gao, D., Zhang, Y.A. & Gao, W. 2008. Effect of moisture adjustments on vertical temperature distribution during forced-aeration static-pile composting of sewage sludge. *Resources, Conservation and Recycling* **52**, 635–642.

Malinska, K., Zabochnicka-Świątek, M. & Dach, J. 2014. Effects of biochar amendment on ammonia emission during composting of sewage sludge. *Ecological Engineering* **71**, 474–478.

Marche, T., Schnitzer, M., Dinel, H., Pare, T., Champagne, P., Schulten, H.R. & Facey, G. 2003. Chemical changes during composting of a paper mill sludge-hardwood sawdust mixture. *Geoderma* **116**, 345–356.

Marengo, J.R., Kok, R.A., O´Brien, G.K., Velagaletti, R.R. & Stamm, J.L. 1997. Aerobic biodegradation of (C-14)-sarafloxacin hydrochloride in soil. *Environmental Toxicology and Chemistry* **16**, 462–471.

Martín, J., Camacho-Mu˜noz, M.D., Santos, J.L., Aparicio, I. & Alonso, E. 2012. Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal. *Journal of Hazardous Materials* **239–240**, 40–47.

Martin, J., Luis Santos, J., Aparicio, I. & Alonso, E. 2015. Pharmaceutically active compounds in sludge stabilization treatments: Anaerobic and aerobic digestion, wastewater stabilization ponds and composting. *Science of the Total Environment* **503**, 97–104.

Maulini-Duran, C., Artola, A., Font, X. & Sánchez, A. 2013. A systematic study of the gaseous emissions from biosolids composting: Raw sludge versus anaerobically digested sludge. *Bioresource Technology* **147**, 43–51.

Menert, A., Kriipsalu, M., Pihlap, E., Anderson, K., Orupõld, K., Toomik, E. & Sipp Kulli, S. 2014. Study on biodegrading microbiota of wood residues. Abstract Book, *2nd Congress of Baltic Microbiologists*, Tartu, 16–19 October 2014.University of Tartu.

Michelini, L., Reichel, R., Werner, W., Ghisi, R. & Thiele-Bruhn, S. 2012. Sulfadiazine Uptake and Effects on Salix fragilis L. and Zea mays L. Plants. *Water, Air & Soil Pollution* **223**, 5243–5257.

Mollazadeh, N. 2014. Composting: a new method for reduction of solid waste and wastewater. *International Research Journal of Applied and Basic Sciences* **8**, 311–317.

Montforts, M.H.M.M. 2005. The trigger values in the environmental risk assessment for (veterinary) medicines in the European Union: a critical appraisal. RIVM report 601500002, p. 37.

Motoyama, M., Nakagawa, S., Tanoue, R., Sato, Y., Nomiyama, K. & Shinohara, R. 2011. Residues of pharmaceutical products in recycled organic manure produced from sewage sludge and solid waste from livestock and relationship to their fermentation level. *Chemosphere* **84**, 432–438.

Narumiya, M., Nakada, N., Yamashita, N. & Tanaka, H. 2013. Phase distribution and removal of pharmaceuticals and personal care products during anaerobic sludge digestion. *Journal of Hazardous Materials* **260**, 305–312.

Nayak, A.K. & Kalamdhad, A.S. 2014, Feasibility of Composting Combinations of Sewage Sludge, Cattle Manure, and Sawdust in a Rotary Drum Reactor. *Environmental Engineering Research* **19**, 1–11.

Nei, L., Haiba, E., Kutti, S., Lillenberg, M., Ivask, M., Peda, J. & Kuu, A. 2015. Pharmaceuticals and the quality of sewage sludge compost. Abstracts Book: *2015 International Composting Conference.* Beijing, China, 14.

Nei, L., Lillenberg, M., Haiba, E., Kipper, K. & Herodes, K. 2010. Sewage Sludge – a Nutrient-Rich Fertilizer or Hazardous Waste. *In: Innovative Approaches and Sustainable Technology*, Kohtla-Järve, pp. 17–21.

Nei, L., Haiba, E., Kutti, S., Kipper, K., Herodes, K. & Lillenberg, M. 2014. Sewage sludge compost, microbial activity and pharmaceuticals. *Global Journal on Advances in Pure & Applied Sciences* **3**, 30–37.

Nouri, J., Mahvi, A.H., Jahed, G.R. & Babaei, A.A. 2008. Regional distribution pattern of groundwater heavy metals resulting from agricultural activities. *Environmental Geology* **55**, 1337–1343.

Pae, T., Luud, A. & Sepp, M. 2005. Artificial mountains in North-East Estonia: monumental dumps of ash and semi-coke. *Oil Shale* **22**, 333–344.

Pankhurst, C.E., Doube, B.M. & Gupta, V.V.S.R. 1997. Biological Indicators of Soil Health: Synthesis. In: Pankhurst C.E., Doube B.M., Gupta V.V.S.R (eds). *Biological Indicators of Soil Health*, pp. 419–435.

Pare, T., Dinel, H., Schnitzer, M. & Dumontet, S. 1998. Transformations of karbon and nitrogen during composting of animal manure and shredded paper. *Biology and Fertility of Soils* **26**, 173–178.

Peda, J. & Kutti, S. 2013. Decomposition of bioplastic bags under different environmental conditions of vermicomposters. Вермикомпостирование и вермикультивирование как основа экологического земледелия в xxi веке – проблемы, перспективы, достижения: Vermicomposting and Vermiculture as basis of ecological landownership in XXI century – problems, outlooks, achievements, Minsk, pp 168−172.

Peysson, W. & Vulliet, E. 2013. Determination of 136 pharmaceuticals and hormones in sewage sludge using quick, easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography-time-of-flight-mass spectrometry. *Journal of Chromatography A* **1290**, 46–61.

Platen, H. & Witrz, A. 1999. Application of analysis no 1: Measurement of the respiration activity of soils using the OxiTop® Control measuring system. *Basic principles and process characteristic quantities.* Wissenschaftlich- Technische Werkstätten GmbH & Co. Germany.

Prosser, R.S. & Sibley, P.K. 2015. Review- Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. *Environment International* **75**, 223–233.

Raud, M., Kesperi, R., Oja, T., Oltand, J. & Kikas, T. 2014. Utilization of urban waste in bioethanol production: potential and technical solutions. *Agronomy Research* **12**, 397–406.

Redshaw, C.H., Wootton, V.G. & Rowland, S.J. 2008. Uptake of the pharmaceutical Fluoxetine Hydrochloride from growth medium by Brassicaceae. *Phytochemistry* **69**, 2510–2516.

Reichel, R., Rosendahl, I., Peeters, E.T.H.M., Focks, A., Groeneweg, J., Bierl, R., Schlichting, A., Amelung, W. & Thiele-Bruhn, S. 2013. Effects of slurry from sulfadiazine (SDZ) and difloxacin (DIF) medicated pigs on the structural diversity of microorganisms in bulk and rhizosphere soil. *Soil Biology & Biochemistry* **62**, 82–91.

Richardson, M.L. & Bowron, J.M. 1985. The fate of pharmaceutical chemicals in the aquatic environment – a review. *Journal of Pharmacy and Pharmacology* **37**, 1–12.

Rivera-Utrilla, J., Sánchez-Polo, M., Ferro-García, M.A., Prados-Joya, G. & Ocampo-Pérez, R. 2013. Pharmaceuticals as emerging contaminants and their removal from water. A review. *Chemosphere* **93**, 1268–1287.

Rodale, J.I. 1960. The complete book of composting, Publisher: Emmaus, Pa.: Rodale Books, 1960.

Rodríguez, L., Cerrillo, M.I., García-Albiach, V. & Villaseñor, J. 2012. Domestic sewage sludge composting in a rotary drum reactor: Optimizing the thermophilic stage. *Journal of Environmental Management* **112**, 284–291.

Rodríguez-Rodríguez, C.E., Jelic´, A., Llorca, M., Farré, M., Caminal, G., Petrovic´, M., Barceló, D. & Vicent, T. 2011. Solid-phase treatment with the fungus Trametes versicolor substantially redudes pharmaceutical concentrations and toxicity from sewage sludge. *Bioresource Technology* **102**, 5602–5608.

Rodríguez-Rodríguez, C.E., Barón, E., Gago-Ferrero, P., Jelic, A., Llorca, M., Farré, M., Díaz-Cruz, M.S., Eljarrat, E., Petrovic, M., Caminal, G., Barceló, D. & Vicent, T. 2012. Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus Trametes versicolor in bioslurry reaktor. *Journal of Hazardous Materials* **233–234**, 235–243.

Ruggieri, L., Gea, T., Mompeo, M., Sayara, T. & Sanchez, A. 2008. Performance of different systems for the composting of the source-selected organic fraction of municipal solid waste. *Biosystems Engineering* **101**, 78–86.

Shaheen, S.M., Shams, M.S., Elbehiry, F.A. & Ibrahim, S.M. 2012. Organic Amendments and Earthworm Addition Improve Properties of Nonacidic Mine Tailings. *Applied and Environmental Soil Science* **2012**, 1–11.

Singh, R.P. & Agrawal, M. 2010. Variations in heavy metal accumulation, growth and yield of rice plants grown at different sewage sludge amendment rates. *Ecotoxicology and Environmental Safety* **7**, 632–641.

Sinha, R.K., Patel, U., Soni, B.K. & Li, Z. 2014. Earthworms for safe and useful management of solid wastes and wastewaters, remediation of contaminated soils and restoration of soil fertility, promotion of organic farming and mitigation of global warming: A review. *Journal of Environment and Waste Management* **1**, 011–025.

Smith, M., Friend, D. & Johnson, H. 2007. Composting for the homeowner, University of Illinois Extension, *http://web.extension.illinois.edu/homecompost/science.html.*

State Agency of Medicines. 2011. Estonian Statistics on Medicines 2006–2010, Published by: Ravimiamet, p. 79 (in Estonian).

State Agency of Medicines. 2013. Estonian Statistics on Medicines 2013, p 46 (in Estonian).

Statistics Estonia. 2006. Agriculture Yearbook, Statistical Office of Estonia, Estonia, p. 64 (in Estonian).

Statistics Estonia. 2012. Agriculture, Statistical Yearbook of Estonia, Statistical Office of Estonia, Estonia, pp. 273–282 (in Estonian).

Stumpf, M., Ternes, T.A., Wilken, R-D., Rodrigues, S.V. & Baumann, W. 1999. Polar drug residues in sewage and natural waters in the State of Rio de Janeiro, Brazil. *Science of the Total Environment* **225**,135–141.

Torri, S.I., Corrêa, R.C., Renella, G., Valdecantos, A. & Perelomov, L. 2012. Biosolids Soil Application: Why a New Special on an Old Issue? *Applied and Environmental Soil Science* **2012**, 1–3.

Torri, S.I., Corrêa, R.S., Renella, G., Perelomov, L. & Valdecantos, A. 2014. Biosolids Soil Application: Agronomic and Environmental Implications 2013. *Applied and Environmental Soil Science* **2014**, 1–3.

Van Doorslaer, X., Dewulf, J., Van Langenhove, H. & Demeestere, K. 2014. Fluoroquinolone antibiotics: an emerging class of environmental micropollutants. *Science of the Total Environment* **500–501**, 250–269.

Varnik, R., Karp, K., Pihelgas, E., Merivee, A. & Lehtsaar, J. 2006. Effect of semicoke-based compost mixtures on gross margin of horticultural crops. *Oil Shale* **23**, 39–52.

Vasskog, T., Bergersen, O., Anderssen, T., Jensen, E. & Eggen, T. 2009. Depletion of selective serotonin reuptake inhibitors during sewage sludge composting. *Waste Management* **29**, 2808–2815.

Walters, E., McClellan, K. & Halden, R.U. 2010. Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. *Water Research* **44**, 6011–6020.

Wang, Q., Wang, H., Sun, B., Bai, J. & Guan, X. 2009. Interactions between oil shale and its semi-coke during co-combustion. *Fuel* **88**, 1520–1529.

Wang, X., Cui, H., Shi, J., Zhao, X., Zhao, Y. & Wei, Z. 2015. Relationship between bacterial diversity and environmental parameters during composting of different raw materials. *Bioresource Technology* **198**, 395–402.

Wang, H., Brown, S.L., Magesan, G.N., Slade, A.H., Quintern, M, Clinton, P.W. & Payn, T.W. 2008. Technological options for the management of biosolids. *Environmental Science and Pollution Research* **15**, 308–317.

White, P.W., Crawford, J.W., Álvarez, M.C.D. & Moreno, R.G. 2013. Soil Management for Sustainable Agriculture 2013. *Applied and Environmental Soil Science* **2014**, 1–2.

White, R.E., Torri, S.I. & Corrêa, R.S. 2011. Biosolids Soil Application: Agronomic and Environmental Implications. *Applied and Environmental Soil Science* **2011**, 1–3.

Wu, X., Dodgen, L.K., Conkle, J.L. & Gan, J. 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. *Science of ehe Total Environment* **536**, 655–666.

Yue, B., Chen, T.-B., Gao, D., Zheng, G.-D., Liu, B. & Lee, D.-J. 2008. Pile settlement and volume reduction measurement during forced-aeration static composting. *Bioresource Technology* **99**, 7450–7457.

Zhang, Y., Geißen, S.-U. & Gal, C. 2008. Review. Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere* **73**, 1151–1161.

Zhou, H.-B., Ma, C., Gao, D., Chen, T.-D., Zheng, G.-D., Chen, J. & Pan, T.-H. 2014. Application of a recyclable plastic bulking agent for sewage sludge composting. *Bioresource Technology* **152**, 329–336.

Zorpas, A.A. & Loizidou, M. 2008. Sawdust and natural zeolite as a bulking agent for improving quality of a composting product from anaerobically stabilized sewage sludge. *Bioresource Technology* **99**, 7545–7552.

Zuloaga, O., Navarro, P., Bizkarguenaga, E., Iparraguirre, A., Vallejo, A., Olivares, M. & Prieto, A. 2012. Overview of extraction, clean-up and detection techniques for the determination of organic pollutants in sewage sludge: A review. *Analytica Chimica Acta* **736**, 7–29.

PAPER V

Nei, L., **Haiba, E**., Kutti, S., Lillenberg, M., Kipper, K., Herodes, K. (2014). Sewage sludge compost, microbial activity and pharmaceuticals. New Trends and Issues Proceedings on Advances in Pure and Applied Sciences, *Global Journal on Advances Pure and Applied Sciences,* North America, 312 09 2014, 30−37.

Global Journal on Advances in Pure & Applied Sciences

Vol 3 (2014) 30-37

Selected Paper of 2nd Global Conference on Environmental Studies (CENVISU-2014), 09-10 April 2014, Quality Hotel Rouge et Noir Conference Center, Rome, Italy

Sewage sludge compost, microbial activity and pharmaceuticals

Lembit Nei*, Tartu College, Tallinn University of Technology, Puiestee, Tartu, Estonia.
Egge Haiba, Tartu College, Tallinn University of Technology, Puiestee, Tartu, Estonia.
Sander Kutti, Institute of Chemistry, University of Tartu, Ravila, Estonia.
Karin Kipper, Institute of Chemistry, University of Tartu, Tartu, Estonia.
Koit Herodes, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi, Estonia.
Merike Lillenberg, Department of Environmental Science, Policy and Geography, University of South Florida, USA.

Suggested Citation:
Nei, L., Haiba, E., Kutti, S., Kipper, K., Herodes, K. & Lillenberg, M. Sewage sludge compost, microbial activity and pharmaceuticals, *Global Journal on Advances in Pure & Applied Sciences* [Online]. 2014, 03, pp 30-37. Available from: http://www.world-education-center.org/index.php/paas

Received November 03, 2013; revised February 20, 2014; accepted March 23, 2014.
Selection and peer review under responsibility of Dr. Nehir Varol, Near East University, Cyprus.

Abstract

Sewage sludge compost has to be contaminant-free to ensure safe land application. However, it may contain substantial amounts of pharmaceuticals, personal care products and microbial pollution. In Estonia sewage sludge is primarly composted and re-used as an agricultural ferilizer. The aim of the current study was to determine the relationship between the concentrations of ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFL), sulfadimethoxine (SDM) and sulfamethoxazole (SMX) and the microbial activity. The pharmaceutical residue can inhibit active and growing microorganisms and therefore have a negative impact on composting processes resulting in poor compost quality, non-inactivated pathogens and high microbial biomass. The concentration of the pharmaceuticals in the sewage sludge during a 120 day composting period was measured by pressurized liquid extraction (PLE), then followed by solid phase extraction (SPE), and high-performance liquid-chromatography - mass spectrometry (LC-MS). Soil microbial respiration rates (basal respiration) were measured

* ADDRESS FOR CORRESPONDENCE: **Lembit Nei,** Tartu College, Tallinn University of Technology, Puiestee 78, 51008 Tartu, Estonia, *E-mail address:* lembit.nei@ttu.ee

using manometric respirometers (Oxitop®, WTW), which allow the determination of the sample oxygen consumption. Substrate induced respiration (SIR) was also determined via the Oxitop® manometric system.
The initial concentration of every pharmaceutical was 2 mg/kg in relation to dry weight (dw). All the pharmaceuticals had a significant decrease in concentration during the composting period. The highest decomposition rates in different sewage sludge composts were measured for sulfonamides (average 93%) and the lowest for fluoroquinolones (average 94%).
Most of the compost piles showed significant decrease after 120 days in both BA and SIR values. After 4 months the basal respiration values of the compost ranged from 0.04-0.16 mgO2/g DW*h. The basal respiration value for the compost pile without pharmaceuticals was 0.26 mgO2/g DW*h. The compost containing pharmaceutical residues showed an average bacterial-to-fungal ratio of 29 : 71 whereas the control compost pile showed a ratio of 65 : 35. That clearly indicates that pharmaceutical residues in a compost pile have a negative impact not only on the microbial activity of the composting process, but also on the microbial community composition.

Keywords: sewage sludge, composting, pharmaceuticals, microbial processes.

1. Introduction

Agricultural application of sewage sludge has become the most widespread method for its disposal, compared to the other methods (as incineration) it is the most economical outlet for sludge [21]. Sewage sludge compost is rich in nutrients and trace elements and could be re-used in agriculture as fertilizer and for soil as stimulating its biological activity [12]. Nowadays more than 60 % in the U.S. and 40 % in Europe of the sewage sludge produced are applied to the land [7, 21].

Fertilizing soil with sewage sludge compost a large quantities of different drug residues have been detected in terrestrial environment [8, 16, 15]. Increasing amounts of pharmaceuticals and their metabolites reach wastewater treatment plants mainly through excreta and disposal of unused or expired drugs [13].

Sewage and its sludge is known to contain pharmaceuticals [19, 10, 11, 3, 9, 21]. Since sewage sludge is primary composted in Estonia and often re-used in agriculture as ferilizer, the pharmaceutical residue can inhibit active and growing microorganisms and therefore have a negative impact on composting processes resulting in poor compost quality, non-inactivated pathogens and high microbial biomass. Antibiotics may inhibit key composting processes mediated by microorganisms even at low doses [1].

Sewage treatment facilities do not remove all pharmaceutical residues completely and several antimicrobials do not decompose during sewage sludge composting process [13, 10, 11, 5]. Antibiotics are designed to be refractory to biodegradation and to act effectively even at low doses [1]. The sewage sludge containing pharmaceutical residues is used as a fertilizer reach the soil where they can affect microorganisms, accumulate in plants and may have adverse effects on living organisms [11]. Some of the antibiotics frequently found in agricultural soils are bacteriostatic, such as sulfonamides; others are bactericidal, such as fluoroquinolones [18, 11].

To compare the influence of different sewage sludge composting technologies to the degradation rate of the fluoroquinolones and sulfonamides in it - some new experiments were performed. In the current study the selection of pharmaceuticals was made considering their resistance in soil and the scale of their use [1, 10, 9, 14]. The studied pharmaceuticals included fluoroquinolones: ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFL); and sulfonamides: sulfadimethoxine (SDM) and sulfamethoxazole (SMX). Sewage sludge and support material were composted at the presence of antimicrobials for 120 days.

2. Materials and Methods

2.1. Chemicals and materials

Antibiotics — three fluoroquinolones: ciprofloxacin (CIP, purity 99.8%), norfloxacin (NOR, purity 99.9%) and ofloxacin (OFL, purity 99.3%) and two sulfonamides: sulfadimethoxine (SDM, purity 99.4%) and sulfamethoxazole (SMX, purity 99.9%) — and ammonia were purchased from Riedel-de-Haën (Seelze, Germany). Hydrophilic–lipophilic balanced (HLB) cartridges (Oasis HLB (60 µm), 500 mg/6 mL) were supplied by Waters (Milford, MA, USA). 1, 1, 1, 3, 3, 3-hexafluoroisopropanol (HFIP) was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile and methanol were obtained from J.T. Baker (Deventer, The Netherlands), phosphoric acid from Lachema (Brno, Czech Republic) and citric acid monohydrate from Fisher Scientific (Pittsburgh, PA, USA). All chemical analyses were performed by the Institute of Chemistry, University of Tartu.

2.2. Preparation of compost mixtures

The sewage sludge from an Estonian city was used to make four different composts. Sewage sludge composts with different reference substances (Table 1) were used in model experiments under fixed conditions. The initial concentration of every pharmaceutical was 2 mg/kg in relation to dry weight (dw).

Table 1. Descriptions of composts

Compost No	sewage sludge treatment technology	Bulking agent	Added pharmaceuticals in compost
1	methane fermentation	peat	2 mg/kg (dw)
2	methane fermentation + vermicomposting*	sawdust	2 mg/kg (dw)
3	methane fermentation	sawdust + oil-shale ash	2 mg/kg (dw)
4	methane fermentation	sawdust	Not added

**Dendrobaena veneta* were added

The experiment was conducted in a non-heated room with a mean air temperature of 22-25°C. The duration of composting was 120 days. Experiments were performed in non-transparent plastic containers. The temperature of the composting material was measured during the whole experiment. The matter was mixed periodically every 5-7 days to provide both sufficient aeration and homogenization. Water was added depending on the dry matter content of sludge composts.

2.3. Chemical parameters of compost mixtures

The chemical composition of the studied wastes was analysed at the beginning and at the end of the experiment (Table 2). All chemical analyses were performed by the Plant Biochemistry Laboratory of Estonian University of Life Sciences. The methodologies of the analyses are presented in detail in the Official Methods of Analysis (1990). The following applications were carried out: moisture (gravimetric), pHKCl, total nitrogen (TotN, by the Kjeldahl method), total phosphorus (TotP), and potassium (flame photometric method,). The content of organic matter (loss on ignition) was determined according to Schulte, [20].

Table 2. The chemical composition of the composts at the beginning and at the end of the experiment

Compost No		Organic matter, %	Moisture, %	pH	Total N, %	Total P, g/kg	Total K, g/kg
1	beginning	56.1	76.5	6.3	3.0	1.3	0.5
	after 120 days	54.3	70.0	5.8	3.1	1.3	0.4
2	beginning	69.0	74.9	6.8	1.5	1.3	1.0
	after 120 days	55.0	73.5	6.4	2.6	1.7	2.9
3	beginning	47.6	66.3	7.8	1.5	0.6	2.0
	after 120 days	33.9	69.5	7.9	1.6	0.5	2.2
4*	beginning	74.5	74.0	6.8	1.4	1.3	1.3
	after 120 days	62.5	75.4	6.8	2.5	1.4	1.7

* control compost with no additional pharmaceuticals

2.4. Determination of antimicrobials from sewage sludge compost

The methodology used for the determination of antimicrobials from sewage sludge and compost together with method validation is described in detail by Haiba et al. [6]. Method for simultaneous determination of CIP, NOR, OFL, SDM and SMX from sewage sludge compost consisted of 3 parts: pressurized liquid extraction (PLE), solid phase extraction (SPE), and liquid-chromatography - mass spectrometry (LC-MS). Analyses were carried out (1) on fresh compost mixtures, where investigational drugs were not included, (2) on fresh compost mixtures- which included drugs and (3) compost mixtures stored for 4 months.

2.5. Determination of the microbial parameters of sewage sludge compost

Soil microbial respiration rates (basal respiration) were measured using manometric respirometers (Oxitop®, WTW), which allow the determination of the sample oxygen consumption. The principle of the operation was based on the measurement of the pressure difference in the closed vessel system. During respiration, produced CO_2 was bound to an absorber (soda lime pellets), and microbial oxygen consumption resulted in the pressure drop [17]. The samples were incubated for 4 days at 25°C in the dark.

Substrate Induced Respiration (SIR) was also determined via the Oxitop® manometric system. 50 gram of fieldmoist soil was amended with glycose and incubated in a closed vessel at 22°C in the dark for 24 hours. After the incubation the microbial biomass C was calculated.

To determine the microbial to fungal ratio the selective inhibition technique was used. In order to assess the fungal biomass the samples were treated with cyxloheximide (12 mg/g) and glucose (5 mg/g), for the determination of bacterial biomass the samples were treated with streptomycin (6 mg/g) and glucose (5 mg/g). The controls were treated with both inhibitors- cyxloheximide (12 mg/g) and streptomycin (6 mg/g). All the samples were incubated in closed vessels at 22°C in the dark for 24 hours, after which the biomass C was calculated.

All the microbiological analyses were conducted in Tartu College, Tallinn University of Technology.

3. Results and Discussion

"Blind" determinations of pharmaceuticals from the studied mixtures showed that the background concentrations of fluoroquinolones were always above zero (see Table 3). After adding the pharmaceuticals to the sewage sludge and bulking agent mixtures their initial concentrations in dry matter were determined again. In most cases the concentration of each pharmaceutical was below 2 mg/kg. This is probably because the degradation of pharmaceuticals starts immediately after adding them to the compost mixture. Still, some of the concentrations (in Table 3) are above this value, 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 [2, 4, 22].

According to the data presented in Table 4, the level of degradation after 4-month period from the beginning of the experiment was 92 - 95 % for CIP, 82 - 96 % for NOR, 99 - 100 % for OFL, 83 - 100 % for SMX and 76 - 99 % for SDM.

Table 3. Degradation of antimicrobials during 4-month composting period

Compost No	Compost characteristics	AM concentration in sewage sludge compost µg/kg (dw)				
		SMX	SDM	NOR	CIP	OFL
1	fresh	n.d.	n.d.	39	34	6
	fresh, with pharmaceuticals	2232	1783	1520	975	1678
	after 4-months of composting	375	422	157	82	5
2	fresh	n.d.	n.d.	33	n.d.	n.d.
	fresh, with pharmaceuticals	2038	2220	1997	1349	1893
	after 4-months of composting	6	15	88	61	8
3	fresh	n.d.	n.d.	30	34	27
	fresh, with pharmaceuticals	1742	1779	1724	1108	1540
	after 4-months of composting	n.d.	68	319	65	20

*n.d. - not detected

Table 4. The extent of degradation of pharmaceuticals in compost mixtures during 4-month composting period

Compost No	Degradation after 4 months of composting, %				
	SMX	SDM	NOR	CIP	OFL
1	83	76	90	92	100
2	100	99	96	95	100
3	100	96	82	94	99

The microbial parameters in the control compost did not differ significantly from the compost piles with pharmaceuticals at the beginning of the experiment (Table 5). At the end of the experiment the control compost was clearly showing signs of increased microbial activity compared to the composts with pharmaceuticals, which showed a decrease in microbial activity. Reasons for that increase are not certain yet.

Composts number 2 and 3 have similar characteristics to the control compost- the changes in both BA and SIR are quite similar. Compost number 1 differed significantly from other composts in microbial parameters measured. This is probably due to the bulking agent used. All other composts except for no. 1 used sawdust as bulking agent, not peat. Peat, having slightly antimicrobial properties, caused a noticeable decrease in microbial parameters. The lower microbial activity in compost 1 may have resulted in poorer degradation of some pharmaceuticals shown in Table 4.

Table 5. The microbial parameters of fresh and 120 days old compost

Compost No	Duration	SIR, mg biomassC/g dw	BA, mg O_2/g dw*h
1	Fresh	5.55	0.09
	after 120 days	2.54	0.04
2	Fresh	12.92	0.42
	after 120 days	9.89	0.14
3	Fresh	13.17	0.25
	after 120 days	8.98	0.16
4 *	Fresh	8.87	0.2
	after 120 days	13.02	0.26

SIR- substrate induced respiration, BA- basal respiration,
* control compost with no additional pharmaceuticals

The bacterial-to-fungal ratio (Table 6) of the three composts compared to the control shows clearly that the composting bacteria are affected by pharmaceuticals present in the compost. The control compost has a twofold increase in bacterial activity compared to the other three composts. The abundance of bacteria in the control compost could be explained not only by the lack of pharmaceuticals but also the fact that the microbial activity increased instead of decreasing in the control compost. Bacteria are the first and foremost colonizers of compost and greatly affect the SIR and BA values.

Nevertheless, the presence of pharmaceutical residue clearly affected the microbial community composition- it inhibited bacteria and allowed fungi to be the predominant microorganisms in the composting process.

Table 6. The average bacterial-to-fungal ratio at the end of the experiment.

Compost No	Bacterial, %	Fungal, %
1	34.9	65.1
2	42.7	57.4
3	28.8	71.3
4 *	64.9	35.1

* reference compost without added pharmaceuticals

4. Conclusion

The degradation of five antibiotics was evaluated in laboratory scale composting. More than 90% of the total amount of antibiotics decomposed during the 120 days of sewage sludge composting. The sewage sludge and its compost should be analysed for the content of pharmaceuticals of long persistence before using it as a fertilizer. Sewage sludge and compost are not homogenous, despite mixing them many times. If locally high content of antibiotics exists also in compost ready for utilization the antibiotics can reach soil and plants via fertilizing.

Since microorganisms play a key role in composting processes and nutrient turnover even slight changes in microbial activity and community composition due to antimicrobial agents may result in poor compost quality and prolonged time needed for compost stability. Also, the microbial community may reflect the evolution and performance of the composting process thus acting as an indicator of compost maturity.

The presence of antibiotics in our study clearly affected the microbial community composition of the compost that resulted in lower microbial activity values. Antibiotic residues inhibited a large portion of the bacterial community and therefore allowed fungi to be the predominant decomposers.

Acknowledgements

The authors would like to thank Environmental Investment Centre of Estonia for funding this work.

References

[1] Girardi, C., Greveb, J., Lamshöftc, M., Fetzerd, I., Miltnera, A., Schäffere, A., & Kästner, M. (2011). Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities. *Journal of Hazardous Materials, 198*, 22– 30.

[2] Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C., & Giger, W. (2003). Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Environmental Science & Technology, 37,* 3243 – 3249.

[3] Gunnarsdóttira, R., Jenssenb, P., D., Jensena, P., E., Villumsena, A., Kallenborn, R. (2013). A review of wastewater handling in the Arctic with special reference to pharmaceuticals and personal care products (PPCPs) and microbial pollution. *Ecological Engineering, 50*, 76 – 85.

[4] Göbel, A., Thomsen, A., McArdell, C.S., Joss A., & Giger, W. (2005). Occurrence and Sorption Behavior of Sulfonamides, Macrolides, and Trimethoprim in Activated Sludge Treatment. *Environmental Science & Technology, 39,* 3981 – 3989.

[5] Haiba, E., Lillenberg, M., Kipper, K., Astover, A., Herodes, K., Ivask, M., Kuu, A., Litvin, S.V., & Nei, L. (2013a). Fluoroquinolones and sulfonamides in sewage sludge compost and their uptake from soil into food plants. *African Journal of Agricultural Research, 8*(23), 3000 – 3006.

[6] Haiba, E., Nei, L., Lillenberg, M., Kipper, K., & Herodes, K. (2013b). Degradation of some pharmaceuticals during sewage sludge composting. AWERProcedia Advences in Applied Sciences, *Global Journal on Advances Pure and Applied Sciences,* 857 – 862.

[7] Harrison, E.Z., Oakes, S.O., Hysell, M., & Hay, A. (2006). Organic chemicals in sewage sludges. Review. *Science of the Total Environment, 367*, 481–497.

[8] Ho, Y.B., Zakaria, M.P., Latif, P.A., & Saari, N. (2013). Degradation of veterinary antibiotics and hormone during broiler manure composting. Bioresour Technol, Mar; 131, 476 – 84.

[9] Jelic, A.; Gros, M.; Ginebreda, A.; Cespedes-Sa´nchez, R.; Ventura, F.; Petrovic, M.; Barcelo, D . 2011. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Research, 45, pp 1165 – 1176.

[10] Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Sepp, K., Lõhmus, R., & Nei, L. (2009). Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry. *Journal of Chromatography A,* 1216, 5949 – 5954.

[11] Lillenberg, M., Litvin, S., V., Nei, L., Roasto, M., & Sepp, K. (2010). Enrofloxacin and Ciprofloxacin Uptake by Plants from Soil. *Agronomy Research, 8*(1), 807 – 814.

[12] Margesin, R., Cimadom, J., & Schinner, F. (2006). Biological activity during composting of sewage sludge at low temperatures. *International Biodeterioration & Biodegradation, 57*(2), 88 – 92.

[13] Martín, J., Camacho-Mu˜noz, M., D., & Santos, J., L., Aparicio, I., & Alonso, E. (2012a). Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal. *Journal of Hazardous Materials*, 239-240, 40 – 47.

[14] Martín, J., Camacho-Mu˜noz, M.D., Santos, J.L. Aparicio I., & Alonso, E. (2012b). Distribution and temporal evolution of pharmaceutically active compounds alongside sewage sludge treatment. Risk assessment of sludge application onto soils. *Journal of Environmental Management, 102,* 18 –25.

[15] McClellan, K., & Halden, R.U. (2010). Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey. *Water Research, 44*(2), 658 – 668.

[16] Peysson, W.; Vulliet, E. 2013. Determination of 136 pharmaceuticals and hormones in sewage sludge using quick, easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography–time-of-flight-mass spectrometry. Journal of Chromatography A, 1290, pp 46 – 61.

[17] Platen, H., & Wirtz, A., (1999). Measurement of the Respiration Activity of Soils Using the OxiTop. Control Measuring System. Basic Principles and Process Characteristic Quantities. WTW (Wissenschaftlich-Technische Werkstätten. GmbH & Co. KG, Weilheim, Germany.

[18] Reichel, R., Rosendahl, I., Peeters, E.T.H.M., Focks, A., Groeneweg, J., Bierl, R., Schlichting, A., Wulf Amelung, W., & Thiele-Bruhn, S. (2013). Effects of slurry from sulfadiazine (SDZ) and difloxacin (DIF) medicated pigs on the structural diversity of microorganisms in bulk and rhizosphere soil. *Soil Biology & Biochemistry, 62,* 82 – 91.

[19] Rodríguez-Rodríguez, C. E., Jelic´, A., Llorca, M., Farré, M., Caminal, G., Petrovic´, M., Barceló, D., & Vicent. T. (2011). Solid-phase treatment with the fungus Trametes versicolor substantially redudes pharmaceutical concentrations and toxicity from sewage sludge. *Bioresource Technology, 102*, 5602 – 5608.

[20] Schulte, E. E. (1995). Recommended Soil Organic Matter Tests. In: J. T. Sims, & A. Wolf (Eds.), Recommended Soil Testing Procedures for the Northeastern United States (pp. 47-56). Northeast Regional Bulletin 493. Newark, DE: Agricultural Experiment Station, University of Delaware.

[21] Zuloaga, O., Navarroa, P., Bizkarguenagaa, E., Iparraguirrea, A., Vallejoa, A., Olivaresa, M., & Prieto, A. (2012). Overview of extraction, clean-up and detection techniques for the determination of organic pollutants in sewage sludge: A review. *Analytica Chimica Acta, 736*, 7 – 29.

[22] Yang, S.-F., Lin, C.-F., & Lin, A.Y.-C. (2011). Sorption and biodegradation of sulfonamide antibiotics by activated sludge: Experimental assessment using batch data obtained under aerobic conditions. *Water Research, 45,* 3389 – 3397.

PAPER VI

Haiba, E., Nei, L., Lillenberg, M., Kipper, K., Herodes, K. (2013). Degradation of some pharmaceuticals during sewage sludge composting. *Global Journal on Advances Pure and Applied Sciences*, North America, 123 07 2013, 1, 857−862.

Global Journal on Advances in Pure & Applied Sciences

Issue 1 (2013) 827-832

Selected Paper of 1st Global Conference on Environmental Studies (CENVISU-2013), 24-27 April 2013, Zeynep Sentito Hotel, Belek, Antalya, Turkey

Degradation of some pharmaceuticals during sewage sludge composting

Egge Haiba, Tartu College, Tallinn University of Technology, Puiestee 78, Tartu 51008, Estonia.
Lembit Nei *, Tartu College, Tallinn University of Technology, Puiestee 78, Tartu 51008, Estonia.
Merike Lillenberg, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 62, Tartu 51014, Estonia.
Karin Kipper, Institute of Chemistry, University of Tartu, Ravila 14a, Tartu 50411, Estonia.
Koit Herodes, Institute of Chemistry, University of Tartu, Ravila 14a, Tartu 50411, Estonia.

Suggested Citation:
Haiba, E., Nei, L., Lillenberg, M., Kipper, K. & Herodes, K. *Degradation of some pharmaceuticals during sewage sludge composting, Global Journal on Advances in Pure & Applied Sciences [Online].* 2013, 01, pp 827-832. Available from: http://www.world-education-center.org/index.php/paas

Received November 23, 2012; revised January 21, 2013; accepted March 08, 2013.
Selection and peer review under responsibility of Dr. Nehir Varol.

Abstract

The fate of five antibiotics was studied during sewage sludge composting. These pharmaceuticals were fluoroquinolones (ciprofloxacin $C_{17}H_{18}FN_3O_3$, norfloxacin $C_{16}H_{18}FN_3O_3$ and ofloxacin $C_{18}H_{20}FN_3O_4$) and sulfonamides (sulfadimethoxine $C_{12}H_{14}N_4O_4S$ and sulfamethoxazole $C_{10}H_{11}N_3O_3S$). Different composting technologies were applied. The selection of drugs was made considering the extent of consumption, resistance in soil and the results of plant uptake studies. The presence of these substances in sewage sludge and possible accumulation in plants are acknowledged, but little information is available on their degradation. No systematic work concerning biodegradation of pharmaceuticals when using different sewage sludge composting technologies has been published. This study shows that composting remarkably reduces the concentrations of these pharmaceuticals. In most experiments their concentrations decreased by 95% or more during 4 months of composting. The best results were obtained when the sludge was mixed with sawdust. In the case of using straw or peat instead the decomposition rates were lower.
Keywords: Sewage sludge, composting, pharmaceuticals.

* ADDRESS FOR CORRESPONDENCE: **Lembit Nei**, Tallinn University of Technology, Tartu College, Puiestee 78, Tartu 51008, Estonia. *E-mail address*: lembit.nei@ttu.ee / Tel.: +372-534-58322

1. Introduction

Over the past two decades, the scientific community has become increasingly interested in the impacts of pharmaceutical contaminants to the environment and human health. In contrast to the properties and effects desired from the therapeutic application of antibiotics, these same properties are frequently disadvantageous for those target and non-target organisms present in the environment [1]. The primary route of entry of human pharmaceuticals into the environment is through sewage point sources. Different antimicrobials are often not readily degradable [2; 3]. The sewage sludge and its compost containing drug residues are used as fertilisers in the fields [4-6]. This way drugs reach the soil where they can affect microorganisms and accumulate in plants. The significance of the route involving the uptake of the pharmaceuticals from fertilized soil by plants in terms of risk to human health was shown by Lillenberg *et* al., in 2010 [7].

The concentrations of most of the pharmaceuticals decrease significantly after sewage sludge digestion and compost processes, but many of them are still present in compost [8]. In spite of the fact that even very low drug levels in the environment can have undesirable ecological and health effects, until now the problems related to the presence of pharmaceuticals in sewage sludge and its compost have received little attention. There is only limited information on antibiotics degradation that occurs during sewage sludge composting.

In the current preliminary study model experiments with different compost mixtures were performed with the aim of establishing the impact of the compost composition on the degradation of some pharmaceuticals. These pharmaceuticals included fluoroquinolones and sulfonamides: namely ciprofloxacin (CIP), norfloxacin (NOR) and ofloxacin (OFL), sulfadimethoxine (SDM) and sulfamethoxazole (SMX). The selection of pharmaceuticals in the current study was made considering their wide usage for treatment of human and animal diseases [9], stability in soil and potential accumulation into plants. It has been shown that sawdust can be considered a good bulking agent for use with sewage sludge as it presents the dilution effect on toxic substances [10].

A detailed study performed by Yousefi *et al.* and published in 2013 [11] was to blend one waste high in carbon and low in nitrogen (sawdust) with another waste that is high in nitrogen (municipal solid waste, MSW) in order to obtain an optimum C/N ratio for composting, increase water preservation and improve the final quality of the compost product. The composting piles with 16% and 32% sawdust required shorter composting periods than those without any sawdust. Moreover this study investigated the effect on other quality parameters of compost with different amounts of sawdust added to the raw material (MSW), such as temperature, pH, EC, major cations (Na^+, Mg^{2+}, K^+, and Ca^{2+}), and the heavy metal content of MSW compost. Wong *et al.* have established that addition of coal fly ash significantly reduces the availability of heavy metals in sewage sludge, supporting its use as a co-composting material with sewage sludge [12]. In the present study we tried to find an answer to the question: does the bulking agent have an effect on the degradation of pharmaceutical residues present in sewage sludge compost.

2. Experimental

2.1. Chemicals and materials

Antibiotics and ammonia were purchased from Riedel-de-Haën (Seelze, Germany) — three fluoroquinolones: ciprofloxacin (CIP, purity 99.8%), norfloxacin (NOR, purity 99.9%) and ofloxacin (OFL, purity 99.3%) and two sulfonamides: sulfadimethoxine (SDM, purity 99.4%) and sulfamethoxazole (SMX, purity 99.9%). Hydrophilic–lipophilic balanced (HLB) cartridges (Oasis HLB (60 μm), 500 mg/6 mL) were supplied by Waters (Milford, MA, USA). 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) was obtained from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile and methanol were

obtained from J.T. Baker (Deventer, The Netherlands), phosphoric acid from Lachema (Brno, Czech Republic) and citric acid monohydrate from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Preparation of compost mixtures

Mixtures of sewage sludge, bulking agent and water, containing the studied pharmaceuticals, were composted for four months in insulated containers. The composition of the studied compost mixtures is presented in table 1. The initial concentration of every pharmaceutical was 2 mg/kg in relation to dry matter (dm). The composting piles were mixed periodically every 5-7 days for 4 months to maintain adequate oxygen levels and to homogenize them.

Table 1. The composition of compost mixtures

Pile No	sewage sludge treatment technology	Bulking agent (% from dry matter)	dry matter, %
1	methane fermentation	peat (50)	23.1
2	methane fermentation + vermicomposting*	sawdust (33)	24.7
3	methane fermentation	sawdust + oil-shale ash (29+14)	32.3
4	compressed by centrifugation	sawdust + wood chips (total 43)	25.8
5	compressed by filtration	straw (50)	13.9
6	compressed by filtration + vermicomposting*	sawdust (33)	21.4
7	compressed by filtration	sawdust + oil-shale ash (29+14)	35.5

**Dendrobaena veneta* were added

2.3. Sample preparation and LC-MS analysis

The collected samples were stored at -80^{0}C prior to the preparation and analysis. Pressurized liquid extraction (PLE) with reduced sample size (6±1 g) and solvent volume (20 mL per one cycle) was performed according in-house developed sample preparation method [4]. Solid phase extraction (SPE) procedures are with using hydrophilic-lipophilic balance (HLB) cartridges [4]. The SPE extracts were analyzed by LC–MS (Agilent Series 1100 LC-MSD Trap XCT (Santa-Clara, CA, USA)) equipped with a binary pump, a degasser, an auto-sampler and a column thermostat. For instrument control and data analysis software: Agilent ChemStation for LC Rev. A. 10.02; MSD Trap Control version 5.2 and Data Analysis for LC-MSD Trap 3.2. were used. Antibiotics were chromatographed using a Waters XBridge C18 column (150 mm × 3 mm, 3.5 µm) equipped with a Waters Guard Cartridge (20 mm × 4.6 mm) (Waters, Milford, MA, USA). Gradient elution was performed with methanol and 5 mM HFIP buffer solution (pH 9.0 adjusted with NH_4OH). The linear gradient with a flow rate 0.3 mL/min started at 10% of methanol. The methanol percentage was increased to 55% in 25 minutes, after that methanol content was raised to 100% in 5 minutes and held at 100% for 5 minutes, and then methanol content was lowered to 10% during 5 minutes. For column stabilization the methanol content was held at 10% for 5 minutes. Column temperature was set to 30^{o}C and the injection volume was 10 µL. Antibiotics were detected using electrospray ionization in the positive ion mode and total intensity of fragments selected reaction-monitoring mode detected [5]. Stock solution concentrations were 0.5 mg/mL for SDM and 1 mg/mL for other antibiotics. Stock solutions and working standards in appropriate solvent [7] were stored at -20 ºC. In calibration solutions the concentration of antibiotics ranged from 0.5 ng/mL to 5,000 ng/mL.

3. Results and Discussion

A pilot study of sewage sludge from two Estonian largest cities, Tartu and Tallinn, was performed. Concentrations of NOR, OFL and CIP; SDM and SMX were determined in sewage sludge. In all samples the residues of fluoroquinolones and sulfonamides were present. As a rule, the concentrations of antimicrobials in Tallinn sewage sludge were relatively low. Still, in some cases the contents of CIP, NOR and OFL were over the trigger value set for manure (100 μg/kg). In Tartu, on the contrary, the content of CIP and NOR was mostly over the trigger value, but a high content of OFL was detected only in August, September and October. The content of sulfonamides was quite low in both cities, under the trigger value set for drug residues in manure. The highest concentrations of antibiotics found in Tallinn were: CIP - 1520 μg/kg and NOR - 580 μg/kg (dm). The maximum concentrations of OFL (134 μg/kg), SDM (73 μg/kg) and SMX (22 μg/kg) were lower. The highest concentrations of antimicrobials in sewage sludge of Tartu were: NOR - 439 μg/kg and CIP - 442 μg/kg (dm), OFL - 157 μg/kg (dm), SDM - 32 μg/kg, SMX - 16 μg/kg. These results clearly show that it is important to make sure that the compost made from sewage sludge does not possess any risks originating from the residues of pharmaceuticals.

Small quantities of the studied pharmaceuticals were present in sewage sludge that was used for preparing the compost mixtures used in our experiments. “Blind” determinations of pharmaceuticals from the studied mixtures showed that the background concentrations of fluoroquinolones were never equal to zero (see table 2). This is in agreement with the results of the pilot study concerning the presence of pharmaceuticals in Tallinn and Tartu sewage sludge. After adding the pharmaceuticals to the sewage sludge and bulking agent mixtures their initial concentrations in dry matter were determined again. The results are presented in table 2. In most cases the concentration of each pharmaceutical was below 2 mg/kg. This is probably due to the phenomenon that the degradation of pharmaceuticals starts immediately after adding them to the compost mixture. Still, some of the concentrations (in table 2) are above this value, probably due to the rapid sorption of pharmaceuticals (from liquid phase) to solid particles of sewage sludge or bulking agent. This is in agreement with the data presented in [13]. After 4 months of composting, the concentrations of the formerly added pharmaceuticals were analytically determined again. According to the data presented in table 3, it is evident that the degradation of pharmaceuticals was more complete when sawdust was used as a bulking agent (the degree of degradation of the total amount of pharmaceuticals was 94–98%), if compared to the sewage sludge mixtures with peat and straw (with 88% indicating the extent of degradation). There is no clear proof that the addition of oil-shale ash influences the degradation rate of the studied pharmaceuticals. As huge amounts of coal and oil-shale ash are produced every year and these wastes perform several good qualities as co-composting materials with sewage sludge, it would be reasonable to direct further studies on the establishment of the optimum composition of the sewage sludge compost with sawdust and oil-shale ash as co-composting agents.

4. Conclusions

The degradation of fluroquinolones and sulfonamides takes place during sewage sludge co-composting with sawdust, peat and straw. Additions of sawdust clearly speed up this process, whereas the mixtures with peat and straw perform lower abilities to decompose pharmaceutical residues. No clear evidence was received concerning the impact of vermicomposting and oil shale amendments on the degradation speed of the studied pharmaceuticals.

Table 2. Degradation of pharmaceuticals in different compost samples

Pile No	Sample	pharmaceuticals in dry matter, mg/kg				
		SMX	SDM	NOR	CIP	OFL
1	fresh	0.00	0.00	0.04	0.03	0.01
	fresh, with pharmaceuticals	2.23	1.78	1.52	0.98	1.68
	after 4-months composting	0.38	0.42	0.16	0.08	0.01
2	fresh	0.00	0.00	0.03	0.00	0.00
	fresh, with pharmaceuticals	2.04	2.22	2.00	1.35	1.89
	after 4-months composting	0.01	0.02	0.09	0.06	0.01
3	fresh	0.00	0.00	0.03	0.03	0.03
	fresh, with pharmaceuticals	1.74	1.78	1.72	1.11	1.54
	after 4-months composting	0.00	0.07	0.32	0.07	0.02
4	fresh	0.00	0.00	0.15	0.17	0.05
	fresh, with pharmaceuticals	2.11	1.37	2.33	2.31	3.12
	after 4-months composting	0.01	0.02	0.21	0.04	0.43
5	fresh	0.00	0.03	0.29	0.09	0.12
	fresh, with pharmaceuticals	1.85	1.91	1.56	1.56	1.46
	after 4-months composting	0.02	0.06	0.38	0.16	0.41
6	fresh	0.00	0.00	0.07	0.04	0.02
	fresh, with pharmaceuticals	2.50	2.09	1.58	1.44	0.74
	after 4-months composting	0.02	0.04	0.15	0.05	0.02
7	fresh	0.00	0.00	0.02	0.01	0.00
	fresh, with pharmaceuticals	1.88	1.38	1.61	1.34	1.67
	after 4-months composting	0.01	0.02	0.02	0.01	0.00

Table 3. The extent of degradation of pharmaceuticals in compost mixtures during 4-months composting period

	Degradation after 4 months of composting, %				
Pile No	SMX	SDM	NOR	CIP	OFL
1	83	76	90	92	100
2	100	99	96	95	100
3	100	96	82	94	99
4	100	99	91	98	86
5	99	97	79	90	74
6	99	98	91	97	98
7	99	98	99	100	100

Acknowledgements

The authors would like to thank Environmental Investment Centre of Estonia and Tallinn University of Technology for funding this work (grant B02), Malle Kuum for conducting analysis and Andre Litvin for language corrections.

Haiba, E., Nei, L., Lillenberg, M., Kipper, K. & Herodes, K. *Degradation of some pharmaceuticals during sewage sludge composting, Global Journal on Advances in Pure & Applied Sciences [Online].* 2013, 01, pp 827-832. Available from: http://www.world-education-center.org/index.php/paas

References

[1] Lillenberg, M. Yurchenko, S. Kipper, K. Herodes, K. Pihl, V. Lõhmus, R. Ivask, M. Kuu, A. Kutti, S. Litvin, S.V. & Nei, L. (2010). Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting, *International Journal of Environmental Science and Technology*, 7, 307-312.

[2] Hamscher, G., Sczesny, S. Höper, H., & Nau, H. (2002). Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry, *Analytical Chemistry*, *74*, 1509-1518.

[3] Carballa, M. Omil, F. Lema, J.M. Llompart, M. García-Jares, C. Rodríguez, I. Gómez, M., & Ternes, T. (2004). Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant, *Water Research*, 38, 2004, pp 2918-2926.

[4] Lillenberg, M. Yurchenko, S. Kipper, K. Herodes, K. Pihl, V. Sepp, K. Lõhmus, R., & Nei, L. (2009). Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry, *Journal of Chromatography A*, 1216, 5949-5954.

[5] Lillenberg, M., Herodes, K., Kipper, K., & Nei, L. (2010). Plant Uptake of some Pharmaceuticals from Fertilized Soils, *Proceedings of International Conference on Environmental Science and Technology, Bangkok, Thailand*, 161-165.

[6] Lapen, D.R., Topp, E., Metcalfe, C.D. Li, H. Edwards, M. Gottschall, N., Bolton, P., Curnoe, W., Payne, M., & Beck, A. (2008). Pharmaceutical and personal care products in tile drainage following land application of municipal biosolids, *Science of the Total Environment*, *399,* 50-65.

[7] Lillenberg, M. Litvin, S.V. Nei, L. Roasto, M. & Sepp, K. (2010). Enrofloxacin and Ciprofloxacin Uptake by Plants from Soil, *Agronomy Research*, *8*, 807-814.

[8] Martin, J. Camacho-Munoz, M.D. Santos, J.L. Aparicio, I. & Alonso, E. Distribution and temporal evolution of pharmaceutically active compounds alongside sewage sludge treatment. Risk assessment of sludge application onto soils, *Journal of Environmental Management*, 102, 2012, pp 18-25.

[9] Kwon, J.-W. (2011). Mobility of Veterinary Drugs in Soil with Application of Manure Compost, *Bulletin of Environmental Contamination and Toxicology*, *87*, 40-44.

[10] Banegas, V., Moreno, J.L., Moreno, J.I., García, C., León, G., & Hernández, T. (2007). Composting anaerobic and aerobic sewage sludges using two proportions of sawdust, *Waste Management*, 27, 2007, pp 1317-1327.

[11] Yousefi, J., Younesi, H., & Ghasempoury, S. M. (2013). Co-composting of Municipal Solid Waste with Sawdust: Improving Compost Quality, *Clean Soil Air Water*, *41*, 185–194.

[12] Wong, J.W.C., Fang, M., Li, G.X. & Wong, M.H. (1997). Feasibility of using coal ash residues as co-composting materials for sewage sludge, *Environmental Technology*, *18*, 563-568.

[13] Yang, S.-F. Lin, C.-F. Lin, A.Y.-C. & Hong, P.-K.A. (2011). Sorption and biodegradation of sulfonamide antibiotics by activated sludge: Experimental assessment using batch data obtained under aerobic conditions, *Water Research*, *45*, 3389-3397.

PAPER VII

Haiba, E., Lillenberg, M., Kipper, K., Astover, A., Herodes, K., Ivask, M., Kuu, A., Litvin, S.V., Nei, L. (2013). Fluoroquinolones and sulfonamides in sewage sludge compost and their uptake from soil into food plants. *African Journal of Agricultural Research*, 8 (23), 3000−3006.

academicJournals

Vol. 8(23), pp. 3000-3006, 20 June, 2013
DOI: 10.5897/AJAR12.1539
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

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.

Accepted 6 June, 2013

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-Haėn (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-Haėn, 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

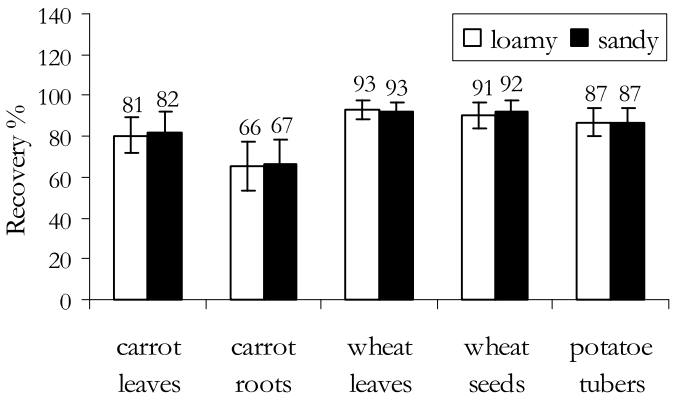

Figure 1. Average recoveries for 5 antimicrobials (CIP, NOR, OFL, SDM, SMX) detected from different parts of food plants grown in different soils using LE and SPE. Error bars show the recovery ranges

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 N2 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 NH4OH).

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 NH4OH). 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.

ACKNOWLEDGEMENTS

This study was funded by the Estonian Environmental Investment Centre, Estonian Science Foundation (grant no 7127), Estonian research target project SF 0180058s07 and Tallinn University of Thechnology. The authors would like to thank Andre Litvin for correcting this manuscript.

REFERENCES

Babel S, Sae-Tang J, Pecharaply A (2009). Anaerobic co-digestion of sewage and brewery sludge for biogas production and land application. Int. J. Environ. Sci. Tech. 6(1):131-140.

Beausse J (2004). Selected drugs in solid matrices: A review of environmental determination, occurrence and properties of principal substances. Trends in Anal. Chem. 23(10-11):753-761.

Boxall ABA, Johnson P, Smith EJ, Sinclair CJ, Stutt E, Levi LS (2006). Uptake of veterinary medicines from soil into plants. J. Agric. Food Chem. 54(6):2288-2297.

Büyüksönmez F, Sekeroglu S (2005). Presence of pharmaceuticals and personal care products (PPCP) in biosolids and their degradation during composting. J. Res. Sci. Technol. 2:31-40.

Carmosini N, Lee LS (2008). Sorption and degradation of selected pharmaceuticals in soil and manure. In: Fate of Pharmaceuticals in the Environment and in Water Treatment Systems. Aga DS (ed). CRC Press, Boca Raton, FL, USA: 139-165.

De Liguoro M, Poltronieri C, Capolongo F, Montesissa C (2007). Use of sulfadimethoxine in intensive calf farming: evaluation of transfer to stable manure and soil. Chemosphere 68(4):671-676.

Dolliver H, Kumar K, Gupta SC (2007). Sulfamethazine uptake by plants from manure-amended soil. J. Environ. Qual., 36(4): 1224-1230.

EMEA/CVMP/055/96 (1998). European Agency for the Evaluation of Medicinal Products. Committee for Veterinary Medicinal Products. Note for guidance: environmental risk assessment for veterinary medicinal products other than GMO-containing and immunological products.

EMEA/MRL/026/95 (1995). Committee for Veterinary Medicinal Products. Sulphonamides (2). Summary report.

EMEA/MRL/820/02 - FINAL (2002). Committee for Veterinary Medicinal Products. Enrofloxacin (extension to all food producing species). Summary report (5).

García-Galán MJ, Díaz-Cruz MS, Barceló D (2009). Combining chemical analysis and ecotoxicity to determine environmental exposure and to assess risk from sulfonamides. Trends Anal. Chem., 28(6):804-819.

Glassmeyer ST, Kolpin DW, Furlong ET, Focazio MJ (2008). Environmental presence and persistence of pharmaceuticals: an overview. In: Fate of Pharmaceuticals in the Environment and in Water Treatment Systems. Aga DS (ed). CRC Press, Boca Raton, London, New York: 3-51.

Hara K, Mino T (2008). Environmental assessment of sewage sludge recycling options and treatment processes in Tokyo. Waste Management, 28(12): 2645-2652.

Hooper DC, Wolfson JS (1991). Fluoroquinolone antimicrobial agents. N. Engl. J. Med. 324(6):384-394.

Isidori M, Lavorgna M, Nardelli A (2005). Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Science of the total environment. 346(1-3):87-98.

Jjemba PK (2002). The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land: a review. Agric. Ecosyst. Environ. 93(1-3):267-278.

Kipper K, Herodes K, Leito I, Nei L (2011). Two fluoroalcohols as components of basic buffers for liquid chromatography electrospray ionization mass spectrometric determination of antibiotic residues. Analyst, 136(21): 4587-4594.

Knapp CW, Dolfing J, Ehlert PAI., Graham DW (2010). Evidence 116 of increasing antibiotic resistance gene abundances in archived soils since 1940. Environ. Sci. Technol. 44(2):580-587.

Lillenberg M, Yurchenko S, Kipper K, Herodes K, Pihl V, Sepp K, Lõhmus R, Nei L (2009). Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid chromatography and electrospray ionization-mass spectrometry. J. Chromatogr. A, 1216(32): 5949-5954.

Lillenberg M, Yurchenko S, Kipper K, Herodes K, Pihl V, Lõhmus R, Ivask M, Kuu A, Kutti S, Litvin SV, Nei L (2010). Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting. Int. J. Environ. Sci. Tech. 7(2):307-312.

Migliore L, Cozzolino S, Fiori M (2003). Phytotoxicity to and uptake of enrofloxacin in crop plants. Chemosphere 52(7):1233-1244.

Montforts MHMM (2005). The trigger values in the environmental risk assessment for (veterinary) medicines in the European Union: a critical appraisal. RIVM report 601500002/2005, P. 37.

Palmada J, March R, Torroella E, Espigol C, Baleri T (2000). Determination of enrofloxacin and its active metabolite (ciprofloxacin) at the residue level in broiler muscle using HPLC with fluorescence detection. References of Euroresidue IV.Veldhoven: 822-826.

Pérez S, Eichhorn P, Aga DS (2005). Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole and trimethoprim at different stages of sewage treatment. Environ. Toxicol. Chem. 24(6): 1361-1367.

Picó Y, Andreu V (2007). Fluoroquinolones in soil - risks and challanges. Anal. Bioanal. Chem. 387(4):1287-1299.

Suthar S, Singh S (2008). Comparison of some novel polyculture and traditional monoculture vermicomposting reactors to decompose organic wastes. Ecol. Eng., 33(3-4): 210-219.

Thiele-Bruhn S (2003). Pharmaceutical antibiotic compounds in soils – a review. Plant Nutr. Soil Sci. 166(2):145-167.

Thiele-Bruhn S, Seibicke T, Schulten H-R, Leinweber P (2004). Sorption of sulfonamide pharmaceutical antibiotics on whole soils and particle-size fractions. J. Environ. Qual. 33(4):1331-1342.

Tremier A, de Guardia A, Massiani C, Paul E, Martel JL (2005). A respirometric method for characterising the organic composition and biodegradation kinetics and the temperature influence on the biodegradation kinetics, for a mixture of sludge and bulking agent to be co-composted. Bioresour. Technol. 96(2):169-180.

APPENDIX B

CURRICULUM VITAE

1. Personal data

Name:	Egge Haiba
Date and place of birth:	15.08.1986, Viljandi
E-mail address:	egge.haiba@ttu.ee

2. Education

Educational institution	Graduation year	Education (field of study/degree)
Tartu College, Tallinn University of Technology	2010	Master Degree
Tartu Kivilinna Gymnasium	2005	Secondary education

3. Language competence/skills (fluent, average, basic skills)

Language	Level
Estonian	Native language
English	High level
Russian	Basic level
Spanish	Basic level

4. Special courses

Period	Educational or other organisation
2014	International Engineering Educator „Ing.Paed.IGIP“ certificate
2009	University of Tartu, Spanish Language Course

5. Professional employment

Period	Organisation	Position
2017–…	Tallinn University of Technology, School of Engineering, Tartu College	Lecturer
2011–2016	Tallinn University of Technology, Tartu College of TUT, Department of Sustainable Technology	Lecturer
2010–2011	Tallinn University of Technology, Tartu College of TUT	Project Manager

6. Research activity and thesis supervised

Completed projects

01.08.11–30.06.12	"Pharmaceutical residues and sewage sludge composting technologies" (KIK11104);
01.12.15–28.02.17	"The degradation efficiency of pharmaceuticals and personal care products in sewage sludge compost, depending on the composition of the compost" (KIK16009).

Supervised dissertations

2016	P. Ojala, Master's Degree "Composting pollutants-containing sewage sludge in a controlled environment, by using sawdust in two proportions", Tallinn University of Technology Tartu College of TUT, (sup) E. Haiba, S. Kutti
2016	M. Tooming, Master's Degree "The use and perspectives of treated sewage sludge in Estonian landscape engineering", Tallinn University of Technology Tartu College of TUT, (sup) E. Haiba
2015	T. Tõnisson, Master's Degree, "Design and building a glass bottle house", Tallinn University of Technology Tartu College of TUT, (sup) L. Leetsaar, E. Haiba
2015	E.Nigul, Master's Degree "Analysis of wastewaterand related problems on the example of Ericsson Estonia",Tallinn University of Technology Tartu College of TUT, (sup) E. Haiba, A. Kuu
2015	M. Keerme, Master's Degree "The treatment efficiency of sedimentary pools on the example of Estonia oil shale mine",Tallinn University of Technology Tartu College of TUT, (sup) A. Kuu, E. Haiba
2014	E. Siida, Master's Degree "Optimization of Virtsu wastewater treatment plant's work process and the reasons of an excessive foaming ",Tallinn University of Technology Tartu College of TUT, (sup) K. Kärmas, E. Haiba
2014	K. Erimäe, Master's Degree "The status and potential environmental impact of the wastewater treatment systems in rural areas of Jõgeva and Tartu county", Tallinn University of Technology Tartu College of TUT, (sup) K. Kärmas, E. Haiba
2013	K. Kõrgmaa, Master's Degree "Recreation of workers physical workability", Estonian University of Life Scienses, (sup) O. Sada, E. Haiba
2012	I. Matsujeva, Master's Degree "Application of oil shale ash in sewage sludge composting". Tallinn University of Technology Tartu College of TUT, (sup) L. Nei, E. Haiba

Academic degree

2010 Master´s Degree „ Sewage sludge treatment and usage perspectives in Estonia“, Tallinn University of Technology Tartu College of TUT, (sup) L. Nei

Publications

1. **Haiba, E.**, Nei, L., Kutti, S., Lillenberg, M., Herodes, K., Ivask, M., Kipper, K., Aro, R., Laaniste, A. (2017). Degradation of diclofenac and triclosan residues in sewage sludge compost. *Agronomy Research*, 15 (2), 395−405.
2. Kipper, K., Lillenberg, M., Herodes, K., Nei, L., **Haiba, E.** (2017). Simultaneous determination of fluoroquinolones and sulfonamides originating from sewage sludge compost. *The Scientific World Journal*, 2017, 8p
3. **Haiba, E.** and Nei, L. (2017). Sewage Sludge Composting and Pharmaceuticals. 7th International Scientific Forum, ISF 2017, 7-9 February 2017, Oxford, UK, *Proceeding. European Scientific Institute*, 114−121.
4. **Haiba, E.** and Lillenberg, M. (2017). Head ja halba ravimitest - ravimijäägid keskkonnas. *Apteeker*, 26−28. *In Estonian*.
5. **Haiba, E.**, Nei, L., Ivask, M., Peda, J., Järvis, J., Lillenberg, M., Kipper, K., Herodes, K. (2016). Sewage sludge composting and fate of pharmaceutical residues – recent studies in Estonia. *Agronomy Research*, 14 (5), 1583−1600.
6. Nei, L., **Haiba, E.**, Lillenberg, M. (2016). Pharmaceuticals and Sewage Sludge Compost. Abstracts book: *International Congress on Water, Waste and Energy Management Rome (Italy). ScienceKNOW Conferences*, 186−187.
7. **Haiba, E.** and Nei, L. (2016). Composting studies in Estonia. Abstract book: 7th SETAC World Congress/SETAC North America 37th Annual Meeting. Orlando, Florida: Setac, 29.
8. Nei, L., **Haiba, E.**, Kutti, S., Lillenberg, M., Ivask, M., Peda, J., Kuu, A. (2015). Pharmaceuticals and the quality of sewage sludge compost. 2015 *International Composting Conference*, Beijing, China.
9. Nei, L., **Haiba, E.**, Kutti, S., Lillenberg, M., Kipper, K., Herodes, K. (2014). Sewage sludge compost, microbial activity and pharmaceuticals. New Trends and Issues Proceedings on Advances in Pure and Applied Sciences, Global Journal on Advances Pure and Applied Sciences, North America, 312 09 2014, 30−37.
10. **Haiba, E.**, Ivask, M., Olle, L., Peda, J., Kuu, A., Kutti, S., Nei, L. (2014). Transformation of nutrients and organic matter in vermicomposting of sewage sludge and kitchen wastes. *Journal of Agricultural Science*, 6 (2), 114−118.
11. **Haiba, E.**, Nei, L., Lillenberg, M., Kipper, K., Herodes, K. (2013). Degradation of some pharmaceuticals during sewage sludge composting. Global Journal on Advances Pure and Applied Sciences, North America, 123 07 2013, 1, 857−862.
12. **Haiba, E**., Lillenberg, M., Kipper, K., Astover, A., Herodes, K., Ivask, M., Kuu, A., Litvin, S.V., Nei, L. (2013). Fluoroquinolones and sulfonamides in

sewage sludge compost and their uptake from soil into food plants. *African Journal of Agricultural Research*, 8 (23), 3000−3006.

13. **Haiba, E.**, Kipper, K., Nei, L., Lillenberg, M., Herodes, K. (2013). Pharmaceuticals in Sewage Sludge Compost. *SETAC 23rd Annual Meeting,* Glasgow. MO017.
14. Nei, L., Lillenberg, M., **Haiba, E.** (2012). Plant uptake of some commonly used pharmaceuticals. Abstract book: *SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting*, 20-24 May 2012, Berlin.
15. **Haiba, E.** and Lillenberg, M. (2012). Degradation of pharmaceuticals in sewage sludge compost. Abstract book: *SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting*, 20-24 May 2012, Berlin.
16. Lillenberg, M., **Haiba, E.**, Nei, L. (2012). Ravimijäägid looduskeskkonnas. *Eesti Loodus*, 71−75. *In Estonian*
17. Nei, L., Lillenberg, M., **Haiba, E.**, E.Kipper, K., Herodes, K. (2011). Pharmaceuticals in Sewage Sludge Compost and Their Uptake from Fertilized Soil by Food Plants. Abstract Book: *Society of Environmental Toxicology and Chemistry North America 32nd Annual Meeting*. Boston, Massachusetts, 241.
18. Lillenberg, M., **Haiba, E.**, Nei, L. (2011). Reoveesette sobivusest põlluväetiseks. *Keskkonnatehnika*, 8, 16−18. *In Estonian*.
19. Kipper, K., Herodes, K., Lillenberg, M., Nei, L., **Haiba, E.**, Litvin, S.V. (2010). Plant Uptake of some Pharmaceuticals Commonly Present in Sewage Sludge Compost. In: *Proceedings of 2nd International Conference on Chemical, Biological and Environmental Engineering* (261−264). IEEE.
20. Nei, L., Lillenberg, M., **Haiba, E.**, Kipper, K., Herodes, K. (2010). Sewage Sludge - a Nutrient-Rich Fertilizer or Hazardous Waste. *Innovative Approaches and Sustainable Technology*: Kohtla-Järve. 17−21.

ELULOOKIRJELDUS

1. Isikuandmed

Ees- ja perekonnanimi: Egge Haiba
Sünniaeg ja- koht: 15.08.1986, Viljandi
Kodakondsus: Eesti
E-posti aadress: egge.haiba@ttu.ee

2. Hariduskäik

Õppeasutus (nimetus lõpetamise ajal)	Lõpetamise aeg	Haridus (eriala/kraad)
Tallinna Tehnikaülikool, Tartu Kolledž	2010	Tehnikateaduste magistri kraad
Tartu Kivilinna Gümnaasium	2005	Keskharidus

3. Keelteoskus

Keel	Tase
Eesti	Emakeel
Inglise	Kõrgtase
Vene	Algtase
Hispaania	Algtase

4. Täiendusõpe

Õppimise aeg	Täiendusõppe korraldaja nimetus
2014	International Engineering Educator „Ing.Paed.IGIP“ sertifikaat
2009	Tartu Ülikool, Hispaania keele algkursus

5. Teenistuskäik

Töötamises aeg	Tööandja nimetus	Ametikoht
2017–…	Tallinna Tehnikaülikool, Inseneriteaduskond, Tartu kolledž	Lektor
2011–2016	Tallinna Tehnikaülikool, Tartu Kolledž, Säästva tehnoloogia õppetool	Lektor
2010–2011	Tallinna Tehnikaülikool, Tartu Kolledž	Projektijuht

6. Teadustegevus

Teauspreemiad ja tunnustused

2013 Tallinna Tehnikaülikooli aasta õppejõud 2013 aukiri

Projektid

01.08.11–30.06.12	Kompostimistehnoloogiate mõju ravimijääkide sisaldusele reoveesettest valmistatud kompostis, KIK11104;
01.12.15–30.11.16	Saasteainete lagundamise efektiivsus reoveesete kompostis sõltuvalt komposti koostisest, KIK16009.

Juhendatud väitekirjad

2016	P. Ojala, magistritöö „Saasteaineid sisaldava reoveesette kompostimine kontrollitud tingimustes, kasutades kahes proportsioonis saepuru“, Tallinna Tehnikaülikool Tartu Kolledž, (juh) E. Haiba, S. Kutti
2016	M. Tooming, magistritöö, Töödeldud reoveesette kasutamine ja perspektiivid Eesti maastikuehituse valdkondades, Tallinna Tehnikaülikool Tartu Kolledž, (juh) E. Haiba
2015	T. Tõnisson, magistritöö, Klaaspudelhoone projekt-ehitus, Tallinna Tehnikaülikool Tartu Kolledž, (juh) L. Leetsaar, E. Haiba
2015	E. Nigul, magistritöö „Reovee ja sellega seonduvate probleemide analüüs Ericsson Eesti AS näitel“, Tallinna Tehnikaülikool Tartu Kolledž, (juh) E. Haiba, A. Kuu
2015	M. Keerme, magistritöö „Settebasseinide puhastus-efektiivsus Estonia põlevkivikaevanduse näitel“, Tallinna Tehnikaülikool, Tartu Kolledž, (juh) A. Kuu, E. Haiba
2014	E. Siida, magistritöö „Virtsu reoveepuhasti tööprotsessi optimeerimine ja liigse vahutamise põhjused“, Tallinna Tehnikaülikool, Tartu Kolledž, (juh) K. Kärmas, E. Haiba
2014	K. Erimäe, magistritöö „Jõgeva- ja Tartumaa haja-asustuspiirkondade reoveekäitlussüsteemide olukord ning võimalikud keskkonnamõjud“, Tallinna Tehnikaülikool Tartu Kolledž, (juh) K. Kärmas, E. Haiba
2013	K. Kõrgmaa, magistritöö „Töötaja füüsilise töövõime rekreatsioon“, Eesti Maaülikool, (juh) O. Sada, E. Haiba
2012	I. Matsujeva, magistritöö „Põlevkivituha mõju reoveesette komposteerimisele“, Tallinna Tehnikaülikool, Tartu Kolledž, (juh) L. Nei, E. Haiba

Kaitstud lõputöö

2010	Magistritöö „Ülevaade reoveesette käitlemisest ja kasutamisperspektiividest Eestis, Tallinna Tehnikaülikool, TTÜ Tartu Kolledž, (juh) L. Nei

DISSERTATIONS DEFENDED AT TALLINN UNIVERSITY OF TECHNOLOGY ON *CIVIL ENGINEERING*

1. **Heino Mölder**. Cycle of Investigations to Improve the Efficiency and Reliability of Activated Sludge Process in Sewage Treatment Plants. 1992.

2. **Stellian Grabko**. Structure and Properties of Oil-Shale Portland Cement Concrete. 1993.

3. **Kent Arvidsson**. Analysis of Interacting Systems of Shear Walls, Coupled Shear Walls and Frames in Multi-Storey Buildings. 1996.

4. **Andrus Aavik**. Methodical Basis for the Evaluation of Pavement Structural Strength in Estonian Pavement Management System (EPMS). 2003.

5. **Priit Vilba**. Unstiffened Welded Thin-Walled Metal Girder under Uniform Loading. 2003.

6. **Irene Lill.** Evaluation of Labour Management Strategies in Construction. 2004.

7. **Juhan Idnurm**. Discrete Analysis of Cable-Supported Bridges. 2004.

8. **Arvo Iital**. Monitoring of Surface Water Quality in Small Agricultural Watersheds. Methodology and Optimization of monitoring Network. 2005.

9. **Liis Sipelgas**. Application of Satellite Data for Monitoring the Marine Environment. 2006.

10. **Ott Koppel**. Infrastruktuuri arvestus vertikaalselt integreeritud raudtee-ettevõtja korral: hinnakujunduse aspekt (Eesti peamise raudtee-ettevõtja näitel). 2006.

11. **Targo Kalamees**. Hygrothermal Criteria for Design and Simulation of Buildings. 2006.

12. **Raido Puust**. Probabilistic Leak Detection in Pipe Networks Using the SCEM-UA Algorithm. 2007.

13. **Sergei Zub**. Combined Treatment of Sulfate-Rich Molasses Wastewater from Yeast Industry. Technology Optimization. 2007.

14. **Alvina Reihan**. Analysis of Long-Term River Runoff Trends and Climate Change Impact on Water Resources in Estonia. 2008.

15. **Ain Valdmann**. On the Coastal Zone Management of the City of Tallinn under Natural and Anthropogenic Pressure. 2008.

16. **Ira Didenkulova**. Long Wave Dynamics in the Coastal Zone. 2008.

17. **Alvar Toode**. DHW Consumption, Consumption Profiles and Their Influence on Dimensioning of a District Heating Network. 2008.

18. **Annely Kuu**. Biological Diversity of Agricultural Soils in Estonia. 2008.

19. **Andres Tolli**. Hiina konteinerveod läbi Eesti Venemaale ja Hiinasse tagasisaadetavate tühjade konteinerite arvu vähendamise võimalused. 2008.

20. **Heiki Onton**. Investigation of the Causes of Deterioration of Old Reinforced Concrete Constructions and Possibilities of Their Restoration. 2008.

21. **Harri Moora**. Life Cycle Assessment as a Decision Support Tool for System optimisation – the Case of Waste Management in Estonia. 2009.

22. **Andres Kask**. Lithohydrodynamic Processes in the Tallinn Bay Area. 2009.

23. **Loreta Kelpšaitė**. Changing Properties of Wind Waves and Vessel Wakes on the Eastern Coast of the Baltic Sea. 2009.

24. **Dmitry Kurennoy.** Analysis of the Properties of Fast Ferry Wakes in the Context of Coastal Management. 2009.

25. **Egon Kivi**. Structural Behavior of Cable-Stayed Suspension Bridge Structure. 2009.

26. **Madis Ratassepp**. Wave Scattering at Discontinuities in Plates and Pipes. 2010.

27. **Tiia Pedusaar**. Management of Lake Ülemiste, a Drinking Water Reservoir. 2010.

28. **Karin Pachel**. Water Resources, Sustainable Use and Integrated Management in Estonia. 2010.

29. **Andrus Räämet**. Spatio-Temporal Variability of the Baltic Sea Wave Fields. 2010.

30. **Alar Just**. Structural Fire Design of Timber Frame Assemblies Insulated by Glass Wool and Covered by Gypsum Plasterboards. 2010.

31. **Toomas Liiv**. Experimental Analysis of Boundary Layer Dynamics in Plunging Breaking Wave. 2011.

32. **Martti Kiisa**. Discrete Analysis of Single-Pylon Suspension Bridges. 2011.

33. **Ivar Annus**. Development of Accelerating Pipe Flow Starting from Rest. 2011.

34. **Emlyn D. Q. Witt**. Risk Transfer and Construction Project Delivery Efficiency – Implications for Public Private Partnerships. 2012.

35. **Oxana Kurkina**. Nonlinear Dynamics of Internal Gravity Waves in Shallow Seas. 2012.

36. **Allan Hani**. Investigation of Energy Efficiency in Buildings and HVAC Systems. 2012.

37. **Tiina Hain**. Characteristics of Portland Cements for Sulfate and Weather Resistant Concrete. 2012.

38. **Dmitri Loginov**. Autonomous Design Systems (ADS) in HVAC Field. Synergetics-Based Approach. 2012.

39. **Kati Kõrbe Kaare**. Performance Measurement for the Road Network: Conceptual Approach and Technologies for Estonia. 2013.

40. **Viktoria Voronova**. Assessment of Environmental Impacts of Landfilling and Alternatives for Management of Municipal Solid Waste. 2013.

41. **Joonas Vaabel**. Hydraulic Power Capacity of Water Supply Systems. 2013.

42. **Inga Zaitseva-Pärnaste**. Wave Climate and its Decadal Changes in the Baltic Sea Derived from Visual Observations. 2013.

43. **Bert Viikmäe**. Optimising Fairways in the Gulf of Finland Using Patterns of Surface Currents. 2014.

44. **Raili Niine**. Population Equivalence Based Discharge Criteria of Wastewater Treatment Plants in Estonia. 2014.

45. **Marika Eik**. Orientation of Short Steel Fibers in Cocrete. Measuring and Modelling. 2014.

46. **Maija Viška**. Sediment Transport Patterns Along the Eastern Coasts of the Baltic Sea. 2014.

47. **Jana Põldnurk**. Integrated Economic and Environmental Impact Assessment and Optimisation of the Municipal Waste Management Model in Rural Area by Case of Harju County Municipalities in Estonia. 2014.

48. **Nicole Delpeche-Ellmann**. Circulation Patterns in the Gulf of Finland Applied to Environmental Management of Marine Protected Areas. 2014.

49. **Andrea Giudici**. Quantification of Spontaneous Current-Induced Patch Formation in the Marine Surface Layer. 2015.

50. **Tiina Nuuter**. Comparison of Housing Market Sustainability in European Countries Based on Multiple Criteria Assessment. 2015.

51. **Erkki Seinre**. Quantification of Environmental and Economic Impacts in Building Sustainability Assessment. 2015.

52. **Artem Rodin**. Propagation and Run-up of Nonlinear Solitary Surface Waves in Shallow Seas and Coastal Areas. 2015.

53. **Kaspar Lasn**. Evaluation of Stiffness and Damage of Laminar Composites. 2015.

54. **Margus Koor**. Water Distribution System Modelling and Pumping Optimization Based on Real Network of Tallinn. 2015.

55. **Mikk Maivel**. Heating System Efficiency Aspects in Low-Energy Residential Buildings. 2015.

56. **Kalle Kuusk**. Integrated Cost-Optimal Renovation of Apartment Buildings toward Nearly Zero-Energy Buildings. 2015.

57. **Endrik Arumägi**. Renovation of Historic Wooden Apartment Buildings. 2015.

58. **Tarvo Niine**. New Approach to Logistics Education with Emphasis to Engineering Competences. 2015.

59. **Martin Thalfeldt**. Total Economy of Energy-Efficient Office Building Facades in a Cold Climate. 2016.

60. **Aare Kuusik**. Intensifying Landfill Wastewater and Biodegradable Waste Treatment in Estonia. 2016.

61. **Mart Hiob**. The Shifting Paradigm of Spatial Planning in Estonia: The Rise of Neighbourhood Participation and Conservation of Built-up Areas through the Detailed Case Study of Supilinn, a Historic Suburb of Tartu City, Estonia. 2016.

62. **Martin Heinvee**. The Rapid Prediction of Grounding Behavior of Double Bottom Tankers. 2016.

63. **Bharat Maharjan**. Stormwater Quantity and Quality of Large Urban Catchment in Tallinn. 2016.

64. **Nele Nutt**. The Restoration of Nationally Protected Estonian Manor Parks in the Light of the Florence Charter. 2017.

65. **Üllar Alev**. Renovation and Energy Performance Improvement of Estonian Wooden Rural Houses. 2017.

66. **Simo Ilomets**. Renovation Need and Performance of Envelopes of Concrete Apartment Buildings in Estonia. 2017.

67. **Argo Kuusik**. Determining Biogas Yield from Industrial Biodegradable Waste. 2017.

68. **Katri Pindsoo**. Spatio-Temporal Changes in the Components of Extreme Water Levels on Estonian Coasts. 2017.