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Pharmaceuticals leaching from bio-solids amended soils

Lakning av läkemedelsrester vid slamspridning

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Department of Aquatic Sciences and Assessments
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Abstract

Biosolids are currently applied on arable land in order to recirculate nutrients from urban areas. Biosolids have been reported to contain organic contaminants such as pharmaceuticals; thereby the impact of spreading of biosolids on the environment has to be investigated. This study aims to investigate the transport of pharmaceuticals in lab-scale soil columns using undisturbed soil of different texture (i.e. clay, loam and loamy sand). The selected pharmaceuticals included ß-blockers (i.e. atenolol, metoprolol, propranolol and sotalol), antidepressants (i.e. amitriptyline, carbamazepine, citalopram, diazepam, fluoxetine, lamotrigine, oxazepam, sertraline, venlafaxine and zolpidem) and antibiotics (i.e. azithromycin, ciprofloxacin, clarithromycin, norfloxacin, ofloxacin, roxithromycin, sulfamethoxazole and trimethoprim). The soil columns with the highest clay content showed highest leaching regarding both relative mass and concentration from 9 out of 12 selected pharmaceuticals. The leaching of the pharmaceuticals (absolute amount) decreased in order of clay > loam > loamy sand. Carbamazepine, metoprolol and oxazepam leached highest (absolute amount) whereas trimethoprim, fluoxetine and citalopram leached the lowest (absolute amount). This indicates that the soil texture affect the leaching behaviour of pharmaceuticals by rapid flow through macropores. A correlation between leaching behaviour and physicochemical properties of pharmaceuticals showed hydrophobicity to be significantly negatively correlated with leaching ($p < 0.05$, spearman correlation). However, the study also concludes that other properties (i.e. adsorption to organic carbon, half-life and water solubility) are important to investigate when assessing the transport of pharmaceuticals in undisturbed soils.

Keywords: Biosolids, pharmaceuticals, leaching, soil texture, correlation analysis, physiochemical properties

Sammanfattning

För att återföra näring från det urbana samhället sprids idag slam från svenska vattenreningsverk på åkermark. Slam har visats innehålla olika miljöstörande ämnen bland annat läkemedel, varvid spridning av slammet är omdebatterad och dess miljöpåverkan bör undersökas. Denna studie syftar till att undersöka transport av läkemedelsrester i ostörda jordar med varierande textur (styv lera, mellan lera och sand). Detta gjordes genom ett laboratorieexperiment där slam innehållande läkemedelsrester spreds på jordkolumner och bevattnades med artificiellt regnvatten. Experimentet undersökte transport av beta-blockerare (atenolol, metoprolol, propranolol och sotalol), antidepressiva (amitriptylin, citalopram, diazepam, fluoxetin, karbamazepin, lamotrigin, oxazepam, sertralin, venlafaxin och zolpidem) och antibiotika (azithromycin, ciprofloxacin, klaritromycin, norfloxacin, ofloxacin, roxithromycin, sulfametoxazol och trimetoprim). Jordar med högst lerinnehåll lakade mest både gällande relativ massa och koncentration för 9 av 12 läkemedel. Lakningen av substanserna (absolut massa) minskade i ordningen styv lera > mellan leran > sand. Karbamazepin, metoprolol och oxazepam lakade mest (absolut massa) medan trimetoprim, fluoxetin och citalopram lakade minst (absolut massa). Resultaten indikerar att marktexturen påverkar lakning då läkemedel transporteras genom makroporer. En korrelation mellan lakning och läkemedelssubstansernas fysiokemiska egenskaper visade att graden av hur hydrofobt en substans är ger signifikant negativ korrelation med lakning ($p < 0.05$, spearman korrelation). Dock är det viktigt att ta hänsyn till andra egenskaper så som adsorption till organiskt kol, halveringstid och vattenlöslighet för att kunna förutsäga transport av läkemedelsrester i mark.

Nyckelord: Slamspridning, läkemedel, lakning, marktextur, korrelations analys, fysiokemiska egenskaper

Table of contents

Abbreviations

AMT	amitriptyline
ATL	atenolol
AZC	azithromycin
CBZ	carbamazepine
CFX	ciprofloxacin
CIT	citalopram
CLC	clarithromycin
DZP	diazepam
FXT	fluoxetine
LTG	lamotrigine
MET	metoprolol
NFX	norfloxacin
OFX	ofloxacin
OZP	oxazepam
PRO	propranolol
RXC	roxithromycin
SOT	sotalol
SMX	sulfamethoxazole
SRT	sertraline
TMP	trimethoprim
VEN	venlafaxine
ZPD	zolpidem

1 Introduction

1.1 Background

In order to ensure recirculation of the limited natural resource phosphorus, the Swedish government has decided that by 2018, 40 % of phosphorous and 10 % nitrogen originating from sewage sludge is to be applied on arable land (Swedish Environmental Protection Agency, EPA, 2013). Consequently, approximately 250 000 tons of dry weight sewage sludge is today annually distributed on Swedish farm fields, approximately 25 % (Swedish EPA, 2013). The Swedish Code of Statues, SNFS (1994:2), based on the European sludge legislation 86/278/EEG, regulates the usage of sludge on arable land (Swedish EPA, 2001). This is done in order to ensure protection of the environment by minimizing the risk of exposure to soil, plants, animals and humans and furthermore to surface and groundwater. The distribution of sludge on arable land follows the guidelines for fertilizer distribution, SJVFS (2004:62), governed by the Swedish Board of Agriculture (Swedish board of Agriculture, 2013). Prior to distribution, the quality of the sludge is controlled according to the certification system Revaq which ensures sanitation of the sludge in order to prevent spreading of salmonella (Svenskt Vatten, 2015). The Revaq-certification also ensures that guideline values are not exceeded with regards to concentrations of heavy metals (Pb, Ni, Cu, Cr, Zn, Cd and Hg) in order to prevent accumulation in soil (Svenskt Vatten, 2015[a]). Leachate water from wastewater treatment plants is also analyzed in order to prevent spreading of perfluorooctane sulfonate (PFOS), polyaromatic hydrocarbons (PAH), phthalates and brominated flame retardants (BDE) with the sludge (Svenskt Vatten, 2015[b]). However, there is today no legislation for guideline values with regards to concentrations of pharmaceuticals in sewage sludge (Svenskt Vatten, 2014; Veolia Vatten, 2014). This despite the fact that several monitoring reports have shown occurrence of a wide range of pharmaceuticals in various concentrations in sludge (Adolfsson-Erici et al. 2005; Lindberg et al. 2005; Helmfrid, I. & Eriksson, C. 2010; Lindberg, et al. 2010; Wahlberg et al. 2010; Fick et al. 2011; Haglund, P. & Olofsson, U. 2012).

In Sweden, there have been several reports stating findings of pharmaceuticals in lakes and surface waters (Remberger et al. 2009; Fick et al. 2010; Wahlberg et al. 2010) and drinking water (Wahlberg et al. 2010). The main source for pharmaceutical residues is effluents from wastewater treatment plants. However, manure and biosolids application has also showed to be a source for pharmaceuticals in groundwater (Kay et al. 2004; Hamscher et al. 2005; Blackwell et al. 2007). It might also lead to transport to different water bodies through leaching and surface runoff (Topp et al. 2008; Lapen et al. 2008; Wu et al. 2010). Furthermore, application of manure and biosolids can result in accumulation of pharmaceuticals in the top soil layer (Tolls, J. 2001; Wu et al. 2010). However, since little is known of the environmental transport of pharmaceuticals in soil after biosolids application further research is needed in this area.

Due to the risk pharmaceuticals may pose to the environment, several studies have been conducted investigating adsorption, desorption and transport of human pharmaceuticals (Kreuzig et al. 2005; Chefetz et al. 2008; Lapen et al. 2008 ; Topp et al. 2008; Wu et al. 2010; Fenet et al. 2012; Schaffer et al. 2012). There have also been studies investigating leaching of veterinary pharmaceuticals from animal manure through soil after manure application (Unold et al. 2009; Engelhardt et al. 2015). There have however up to date not been any studies investigating the natural transport and fate of pharmaceuticals from biosolids through undisturbed soil at environmental relevant concentrations. Nor have there been any studies investigating leaching of pharmaceuticals from Swedish soils. In order to ensure human and environmental safety and minimize the risk of their exposure, there is a need to understand the persistence of pharmaceuticals in the environment. Therefore, further investigation of transport of pharmaceuticals in undisturbed soil after biosolid application is needed.

1.2 Aim

The aim of this study was to investigate the transport pattern of selected pharmaceuticals in a lab-scale soil column leaching experiment using three undisturbed soils of contrasting texture (i.e. clay, loam, loamy sand) with incorporated biosolids. The leaching patterns of the pharmaceuticals were correlated with their physical and chemical properties. This study aims to advance the knowledge of risks associated with applying biosolids contaminated with pharmaceuticals on arable land.

1.3 Limitations of the study

Pharmaceuticals and other organic pollutants can be transported through different pathways in the environment but this study was limited only to investigate lateral leaching and did not include e.g. surface runoff. Processes such as volatility, degradation, cocktail effects, transformation or bioaccumulation are important processes which may affect the transport. These processes are not studied separately in this study.

1.4 Research questions

The study aims to answer the following research questions

- Which effects does the soil texture have on the leaching of pharmaceuticals in soil following biosolids application?

- Are the physical-chemical properties (log P_{ow}, log $D_{ow,}$ M_w, K_{ow}, K_{oc}, DT_{50} and S_w) of the pharmaceuticals be correlated to their leaching pattern?

2 Theory

2.1 Background on selected pharmaceuticals

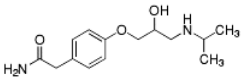
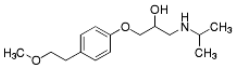
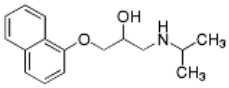
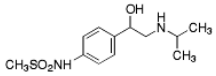
The pharmaceuticals of this study are presented in Table 1. They were selected based on their findings in Swedish sewage sludge (Fick et al. 2011) and effluent water from Swedish wastewater treatment plants (Wahlberg et al. 2010). Amitriptyline (AMT), atenolol (ATL), clarithromycin (CLC), ofloaxcin (OFX), oxazepam (OZP), norfloaxcin (NFX), sulfamethoxazole (SMX), trimethoprim (TMP), zolpidem (ZPD) have been detected in low levels (b.ld. – 100 μg/kg dewatered sludge) in sludge. Carbamazepine (CBZ), citalopram (CIT), ciprofloxacin (CFX), fluoxetine (FXT), metoprolol (MET), sertraline (SRT) and venlafaxine (VEN) were found in high levels (> 100 μg /kg dewatered sludge) (Fick et al. 2011). Atenolol (ATL), metoprolol (MET), oxazepam (OZP), citalopram (CIT) and sulfamethoxazole (SMX) have been found in various concentrations in effluent water from wastewater treatment plants (5 ng/L – 30 ng/L) (Wahlberg et al. 2010).

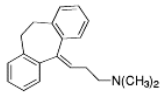
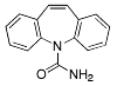
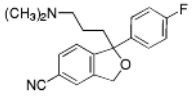
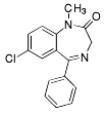
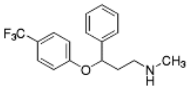
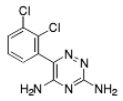
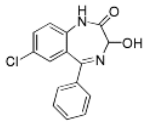
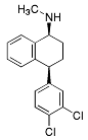
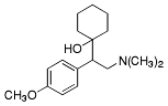
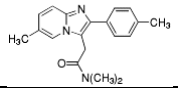
Pharmaceuticals have varying physiochemical properties and functional groups like amines and carboxylic acid. The pharmaceuticals investigated in this study can be clustered in to three classes; ß-blockers, antidepressants and antibiotics (Table 1). They are ionisable compounds that can exist as different ions depending on the pH of the environment (Schwarzenbach et al. 2005). Most of the pharmaceuticals of this study can be characterized as bases due to their high pK_a values (Table 1). DZP, LTG and ZPD are compounds characterized as weak bases and the rest as strong bases (Manallack, D. 2007). ATL, MET, PRO and SOT are characterized as ß-blockers and are strong bases that can exist in cationic or neutral form in the environment (Maszkowska et al. 2014).

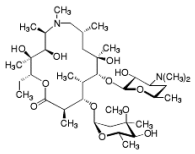
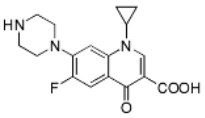
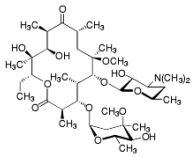
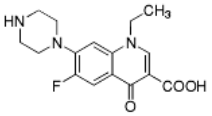
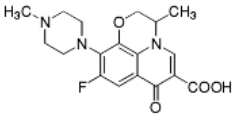
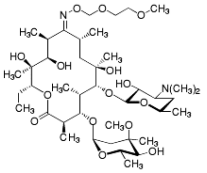
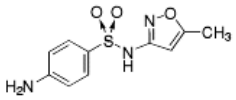
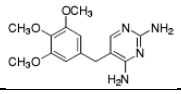
The antidepressants of this study belong to different groups; CIT, FXT and SRT all belong to selective serotonin re-uptake inhibitors (SSRIs) group and have similar modes of action (Gottlieb et al. 2001). VEN belongs to the group selective serotonin and nor-epinephrine re-uptake inhibitor (SSNRI) (Calisto et al. 2009). AMT and CBZ have similar structures and are both tricyclic compounds; however CBZ belong to the group of anticonvulsants as LTG (Muzina et al. 2005). DZP and OZP belong to the group of benzodiazepines with varying usages. ZPD is characterized by being a non-benzodiazepine and is used as a sedative (Calisto et al. 2009).

Of the antibiotics, AZC, CLC and RXC are all macrolides – they are characterized by being large molecules build up by macrocyclic lactone rings containing between 14 to 16 carbon atoms (Dang et al. 2007). CFX, NFX and OFX are fluoroquinolones (FQs) which are zwitterionic compounds meaning that they can be both negatively and positively charged because of their functional groups. SMX belongs to the group sulphonamides and is characterized by two pK_a values. TMP belongs to the group diaminopyrimidine, also characterized by zwitter ionic structures (Thiele-Bruhn, S. 2003).

Table 1. List of pharmaceuticals examined in this study including molecular weight (M_W) in (g/mol), acid dissociation constant (pK_a), octanol-water partitioning constant (log K_{ow}), (soil) organic carbon-water partitioning coefficient (log K_{oc}), water solubility (S_w) in (mg/L), soil half-life values DT_{50} in (days).

Compound	Structure	Chemical formula	M_W	pK_a	Log P_{ow}	Log K_{oc}	S_w	DT_{50}
ß-blockers								
ATL		$C_{14}H_{22}N_2O_3$	266.34[a]	9.6[d]	0.16 [a]	2.17 [c]	13300 [a]	75[q]
MET		$C_{15}H_{25}NO_3$	267.37 [a]	9.6[d]	1.92[h]	1.79 [c]	16900[d]	75[q]
PRO		$C_{16}H_{21}NO_2$	259.35 [a]	9.4 [a]	3.48 [a]	3.09 [c]	61.7 [a]	30[q]
SOT		$C_{12}H_{20}N_2O_3S$	272.37[a]	8.2[h]	0.24 [a]	1.58 [c]	5510 [a]	30[q]

Compound	Structure	Chemical formula	M_W	pK_a	Log P_{ow}	Log K_{oc}	S_w	DT_{50}
Antidepressants								
AMT		$C_{20}H_{23}N$	277.40 [a]	9.4 [a]	4.92 [a]	5.70 [c]	9.71[a]	85[n]
CBZ		$C_{15}H_{12}N_2O$	236.27 [a]	7.0[d]	2.45 [a]	3.59 [c]	17.7 [a]	68[l]
CIT		$C_{20}H_{21}FN_2O$	324.39 [a]	9.59 [f]	3.74 [a]	4.40 [c]	n.d.	360[q]
DZP		$C_{16}H_{13}ClN_2O$	284.74 [a]	3.4 [a]	2.82 [a]	4.05 [c]	50 [a]	75[q]
FXT		$C_{17}H_{18}F_3NO$	309.30 [a]	10.05 [f]	4.05 [a]	5.32 [c]	60.3 [a]	120[q]
LTG		$C_9H_7Cl_2N_5$	256.09 [a]	5.7[g]	2.57[b]	3.13 [c]	170[e]	120[q]
OZP		$C_{15}H_{11}ClN_2O_2$	286.70 [a]	10.9[d]	2.24 [a]	3.08 [c]	179 [a]	54[j]
SRT		$C_{17}H_{17}Cl_2N$	306.23 [a]	9.47 [f]	5.29 [a]	5.53 [c]	3.5[d]	84[m]
VEN		$C_{17}H_{27}NO_2$	277.40 [a]	9.4[d]	3.28 [a]	3.17 [c]	267[c]	120[q]
ZPD		$C_{19}H_{21}N_3O$	307.39 [a]	6.2 [a]	3.85 [a]	4.42[c]	23000[d]	120[q]

Compound	Structure	Chemical formula	M_W	pK_a	Log P_{ow}	Log K_{oc}	S_w	DT_{50}
Antibiotics								
AZC		$C_{38}H_{72}N_2O_{12}$	748.98 [a]	8.7 [a]	4.02 [a]	n.d.	7.09 [a]	71[o]
CFX		$C_{17}H_{18}FN_3O_3$	331.34 [a]	6.16[a]/ 8.63	0.28 [a]	1.55 [c]	30000 [a]	120[q]
CLC		$C_{38}H_{69}NO_{13}$	747.95 [a]	8.9 [a]	3.16 [a]	n.d.	0.342 [a]	n.d.
NFX		$C_{16}H_{18}FN_3O_3$	319.33 [a]	6.1/8.75[d]	0.46[d]	1.97 [c]	178000 [a]	289[o]
OFX		$C_{18}H_{20}FN_3O_4$	361.37 [a]	6.2/9.28[d]	-0.39 [a]	1.65 [c]	28300 [a]	198[o]
RXC		$C_{41}H_{76}N_2O_{15}$	837.05 [a]	9.2[i]	2.75 [a]	n.d.	0.0189[d]	130[p]
SMX		$C_{10}H_{11}N_3O_3S$	253.28 [a]	1.7/5.7[d]	0.89 [a]	3.19 [c]	610 [a]	75[q]
TMP		$C_{14}H_{18}N_4O_3$	290.32 [a]	1.32/7.12[d]	0.91 [a]	2.956[c]	400 [a]	120[q]

Molecular structure from FASS (2015) [a]ChemIDplus Advanced (2015), experimental. [b]Goldstein et al. (2014) [c]ChemSpider. EPISuite (PCKOCWIN v1.66) (2015) [d]Bonnet et al. (2010) [f]Vasskog et al. (2006) [g]Young et al. (2014) [h]Ramil et al. (2009) [i]Kees et al, (2000) [j]Löffler et al. (2005) [l]Carter et al. (2014) [m]Li et al. (2013[a]) [n]Li et al. (2013[b]) [o]Gottschall.et al. (2012) [p]Schlüsener et al. (2006) [q]ChemSpider. EPIISuite. Fugacity model (III) (2015)

2.2 Properties of pharmaceuticals affecting their transport behavior

2.2.1 Physiochemical properties of pharmaceuticals

The physiochemical properties of the pharmaceuticals influences sorption and degradation in sludge and soil, thereby their potential to leach. The octanol-water partitioning coefficient (K_{ow} - P_{ow}) is an indication of the hydrophobicity of a compound. Pharmaceutical compounds with a log $P_{ow} \geq 3$ have been predicted to adsorb strongly to the soil matrix and thus have low potential to leach (Petrie et al. 2014). Compounds with a log $P_{ow} < 1$ are believed to adsorb less strongly to organic matter in the soil and therefore have a higher tendency to leach (Cunningham, V. & Kümmerer, K. (2008). Furthermore, compounds with high water solubility (S_w) (1000 – 10 000 mg/L) have a stronger tendency to leach (U.S. EPA, 2012; Petrie et al. 2014). Pal et al. (2010) showed that compounds with a high molecular weight (M_w) are likely to sorb to sediments and therefore has low leachability.

Many pharmaceuticals contain one or more functional groups making them ionic depending on the pH of the soil and/or solute (Schaffer et al. 2012). Petrie et al. (2014) argues that ionizable pharmaceuticals have been shown to have a strong pH-dependence; therefore the log P_{ow}-concept might give underestimated values a pH-dependent octanol-water coefficient log D_{ow} should be applied. Cunningham, V. & Kümmerer, K. (2008) also states that for large, ionisable substances log P_{ow} should be corrected for log D_{ow} by using equation 1.

$$log\, D_{OW} = log\, K_{OW} + \frac{1}{(1+ 10^{pKa-pH})} \qquad \text{Eq. 1}$$

2.2.2 Ionic strength

When having a carboxylic functional group organic compounds are classified as acidic and when having an amine they are basic. This results in that when $pK_a >$ pH, basic compounds become protonated and thus positively charged, at higher pH the compounds mainly exists in neutral form. At high pH, acidic compounds are deprotonated and therefore negatively charge, at low pH they will mainly exist in non-ionized form (Schwarzenbach et al. 2005; Schaffer et al. 2012; Petrie et al. 2014). Some pharmaceuticals are zwitter ions i.e. they have both acidic and basic functional groups, thereby also two pK_a values (Cunningham, V. & Kümmerer, K. 2008).

2.2.3 Sorption to soil and biosolids

In order to predict the leaching behaviour of pharmaceuticals their adsorption to organic carbon (K_{oc}) can be investigated. However, K_{oc} values are poorly correlated with the K_{ow} values. Tolls, J. (2001) showed that deriving log K_{oc} values from log P_{ow} may lead to underestimation of the log K_{oc} values. Thus log K_{oc} values should be taken with caution but may still give an indication of the sorption behaviour of the pharmaceuticals in soil (Cunningham, V. & Kümmerer, K. 2008).

Compounds with a log K_{oc} 3.5 – 4.4 are classified as compounds with a strong tendency to sorb to soil can be seen as negligible to migrate to ground water (U.S. EPA. 2012). Johnson et al. (2005) stated that compounds with a log $K_{oc} > 3.5$ has a high tendency to sorb to soil and sludge, the study found SRT and FXT to have log K_{oc} values > 3.5 and they were also adsorbed to sludge. The soil and water sorption distribution coefficient (K_d) and sorption to organic carbon is given in equation 2, where $f_{oc, soil}$ represents the fraction of organic carbon in the soil (g/g). The sludge water sorption distribution coefficient (K_p) and sorption to organic carbon is given in Eq 3 where $f_{oc, sludge}$ represents the fraction of organic carbon in the sludge (g/g) (Cunningham, V. & Kümmerer, K. 2008).

$$K_d = K_{oc} \times f_{oc, soil} \qquad \text{Eq. 2}$$

$$K_p = K_{oc} \times f_{oc, sludge} \qquad \text{Eq. 3}$$

2.2.4 Persistence in soil and sludge

Degradation in soil and sludge is an important dissipation pathway for pharmaceuticals. It is dependent on environmental factors such as pH, temperature and the moisture content of the medium. Pharmaceuticals are also sensitive to photolytic degradation (Boxall, A. & Kümmerer, K. 2008). In order to determine degradation, specific degradation rates are commonly used. There are different degradation rates but regarding the degradation in soil DT_{50} is commonly used. Hollis, J. (1991) states non-persistence as $DT_{50} < 5$ days, slightly persistent DT_{50} 5 – 21 days, moderately persistent DT_{50} 22 – 60 days and very persistent $DT_{50} > 60$ days.

2.3 Soil properties influencing leaching behaviour of pharmaceuticals

There are several soil properties that influence the transport behaviour of pharmaceuticals in soils. Kumar et al. (2005) and Golet et al. (2003) found increasing adsorption of antibiotics with increasing soil organic matter and clay content. This shows that sorption soil can be described by other factors apart from K_{oc} as described in the chapter 2.2.3. Soil pH, abundance of metal oxides and cation exchange capacity (CEC) have also shown to affect adsorption, thereby the leaching of pharmaceuticals (Oppel et al. 2004; Schaffer et al. 2012; Srinivasan et al. 2013). Maszkowska et al. (2014) state that pharmaceuticals (e.g. ß-blockers) that are positively charged at low pH are more strongly retained in soil than neutral due to attraction to permanently negatively charged surfaces. Schaffer et al. (2012) states that cationic exchange processes are responsible for retardation of positively charged pharmaceuticals (ATL, DZP and TMP). Also Srinivasan et al. (2013) found sulfonamides to bind more strongly to soils when in cationic form.

However, the above cited studies are all conducted through batch experiments and do not take in to account the structure of the soil. Coarse grained soils have higher water transport capacities and could therefore be predicted to leach more (Eriksson et al. 2005). However, in soils with fine grained material cracks and large biopores can be created through drought and biological activity (Yaron, B. 1989; Eriksson et al. 2005). Solute flow in such macropores can occur with very little interaction with the surrounding soil matrix (Jarvis, N. 2007).Thus, macropores can reduce pharmaceutical sorption to soil surfaces and facilitate rapid transport through the profile and thereby leaching to groundwater. Lapen et al. (2008), Edwards et al. (2009), Larsbo et al. (2009[a]) and Gottschall et al. (2012) have shown that pharmaceuticals may be transported through macropores to tile drains. Kay et al. (2004) also found macroporous transport of veterinary antibiotics in clay after a manure application. D'Alessio et al. (2014) found estrogen being transported in macropores.

2.4 Soil leaching experiments on pharmaceuticals

Undisturbed soil columns have been widely used in research in order to understand the leaching behavior and transport of various contaminants such as pesticides (Larsbo et al. 2013), nutrients (Svanbäck et al. 2013) and metals (Richards et al. 2000) under unsaturated conditions. The main benefits of undisturbed soil column experiments compared to field experiments are that they are easy to control, less costly than and more likely to create an accurate mass balance of the chemical transport (Takamatsu et al. 2007). When conducting column experiments in the laboratory, parameters such as temperature and water content can be kept controlled. Undisturbed column experiments furthermore enable the evaluation of both chemical properties and physical structure on the mobility of the pharmaceuticals (Duran-Álvarez et al. 2014). One benefit of undisturbed soil columns over disturbed is that re-packing of disturbed soil columns eliminates the soil structure and soil macropores. On the other hand, packed columns are homogenized and therefore results in better reproducibility (Lewis, J. & Sjöström, J. 2010).

3 Material and Methods

The soil column leaching experiment and the sample extraction and analysis was performed in the POP laboratory of the Department of Aquatic Sciences and Assessments, SLU, Uppsala, Sweden.

3.1 Materials and chemicals

In this study 22 pharmaceuticals were included; AMT (HCl) (> 95%), ATL (98.5 %), AZC (95 %), CBZ (100 %), CFX (100 %), CIT (HBr) (98 %), CLC (95 %), FXT (HCl) (99,9 %), LTG (> 95%), MET (tartrate salt) (100 %), NFX (> 95%), OFX (99.8 %), PRO (HCl) (99 %), RXC (> 95%), SER (HCl) (98 %), SMX (99.9 %), SOT (HCl) (> 95%), TMP (99.5 %), VEN (HCl) (98 %) were purchased from Sigma-Aldrich. DZP (> 95%), OZP (> 95%) and ZPD (> 95%) (tartrate solution) were acquired as a 1 mg/mL solution, dissolved in an appropriate solvent, from Cerilliant and purchased through Sigma-Aldrich (see Table 1). Of these 22, 17 were included in the spiking solution in the sludge: ATL, AZC, CBZ, CFX, CIT, CLC, DZP, FXT, LTG, MET, OFX, OZP, PRO, SER, SMX, TMP, and VEN.

The isotopically labelled substances carbamazepine-d_{10} (as 100 µg/mL solution), citalopram-d_6 (as 100 µg/mL solution HBr solution, free base), diazepam-d_5 (as 1 mg/mL solution), fluoxetine-d_5 (as 1 mg/mL solution), lamotrigine-^{13}C-$^{15}N_4$ (as 500 µg/mL solution), ofloxacin-d_3, sertraline-d_3 (as 100 µg/mL HCl solution, free base) and venlafaxine-d_6 (as 100 µg/mL HCl solution, free base) were acquired from Sigma-Aldrich. Atenolol-d_7, ciprofloxacin-d_8 and sulfamethoxazole-d_4 were purchased in Toronto Research Chemicals (TRC).

Individual stock standard solutions and isotopically labeled solutions of the substances purchased in solid state were prepared on a weight basis in methanol (at a concentration of 1 mg/mL).

This was done except for CFX, OFX and NFX which were dissolved in methanol adding 100 L of NaOH 1 M. After preparation, standards were stored at −20 ˚C. Intermediate standard solutions, containing all pharmaceuticals, were prepared in methanol, and these solutions were used to prepare working standard solutions. The solvents Acetonitrile (ACN) (99.5 %), Methanol (MeOH) (99.8 %) used in this study were purchased through Merck.

In order to avoid contamination, all glass and metal ware was cleaned according to the following procedure: The glassware was washed with distilled water, rinsed with ethanol three times, dish washed, burned at 400˚C and rinsed with Millipore water three times before use. Plastic material included in the experiment (polyethylene bottles for leachate collection and rainfall simulation, tanks to prepare the artificial rainwater and all PVC material) were washed with distilled water, rinsed with ethanol three times and thereafter rinsed with Millipore water three times.

3.2 Soil sampling

Soil samples were collected from three sites around Uppsala: Rosta Gård, So-1 (59˚59´N; 17˚35´E) and Säby 2, So-2 (59˚50´N; 17˚41´E) and Säby 3, So-3 (59˚54´N; 17˚39´E). The soils were chosen based on their texture characteristics with a clay percentage varying from 4 % to 47 %, see Table 2.

Table 2. Soil properties for sampling sites.

Abbreviation	Texture[a]	Clay (<2 µm) (%)	Silt (2-60 µm) (%)	Sand (60 µm – 2 mm) (%)	pH	OC (%)
So-1	Loamy sand	4.3	12.3	83.4	5.9	1.24
So-2	Loam	19.5	48.2	32.3	5.7	2.45
So-3	Clay	47.3	31.5	21.2	5.8	2.03

[a]U.S Soil Taxonomy Triangle (Pedosphere, 2015)

The characteristics of So-1 were determined by Eurofins and characteristics of So-2 and So-3 at the Soil Physics laboratory at the Department of Soil and Environment, SLU, Uppsala, Sweden. In order to avoid contamination of veterinary pharmaceuticals from stable manure fields that had been conventionally cultivated, thus only received mineral fertilizers were chosen. Prior to sampling, the fields had also received the same mechanical cultivation technique with two carrier cultivations made post-harvest to a depth of 10 – 15 cm. So-1 had been cultivated with rye wheat (*Triticale* spp.) planted during spring 2014 and at So-2 and So-3 with autumn wheat (*Triticum aestivum* spp.).

During the period of November 21, 2014, and December 3, 2014, four undisturbed soil columns (PVC pipes of 20 cm high, 12.5 cm inner diameter) were sampled at each of the three sites. The undisturbed soil columns were obtained according to Larsbo et al. (2013) with the difference that the columns were gently hammered in to the soil and thereafter dug out by hand. The undisturbed columns were collected at least 1.5 m from any edge of the field plot and approximately 1 m apart from each other. Effort was made to avoid tire tracks in order to minimize disturbance due to compaction. Before taking the samples, the upper layer of organic material (top 3 cm) such as plant and biota residues was gently removed (Durán-Álvarez et al. 2014). The columns were prepared by removal of excess soil at the bottom which was done with the help of a sharp spatula. All columns were covered with PVC caps and sealed in plastic bags in order to prevent moisture loss during storage. The columns were stored in a cooling room at + 4 ˚C until the start of the leaching experiments (February 2, 2015), in order to minimize biological activity during storage.

3.3 Sludge sampling

Revaq certified sludge (3 kg of dewatered and thermophilicly digested at 37.3 ˚C) was sampled at Hovgården waste deposit site owned by Uppsala Vatten och Avfall AB on January 15, 2015. The sludge originated from the wastewater treatment plant of Kungsängsverket. The properties of the sludge can be seen in Table 3. The sludge was deposited at Hovgården (59˚93´N; 17˚77´E) during July 2014 and had thus been stored for approximately 6 months prior to sampling. The sludge was frozen at the time of sampling and was therefore chopped in to pieces before packed in plastic containers and stored at +4 ˚C until the homogenizing and spiking procedure January 26, 2015.

Table 3. Properties of dewatered sludge from Hovgården[a].

Average pH	Density (g/m^3)	Average OC (%)
7.5	0.9	8.6

[a]Uppsala Vatten och Avfall AB, (2013)

The sludge was homogenized according to the following procedure. The sludge was placed in a 10 L metal bucket and thereafter broken from large aggregates into small pieces with a pair of sterilized tweezers. Thereafter, the sludge was stirred for approximately 10 minutes, five minutes clockwise and five minutes counter-clockwise in order to ensure complete and even homogenization.

3.4 Column leaching experiment

3.4.1 Sludge spiking

Due to detection purposes in the leachate samples the sludge (1.5 kg) was spiked with 78000 ng absolute of individual pharmaceuticals (i.e. 7800 µL of standard-mix with c = 10 ng/µL) containing the following selected pharmaceuticals: ATL, AZC, CBZ, CFX, CIT, CLC, DZP, FXT, LTG, MET, OFX, OZP, PRO, SER, SMX, TMP, and VEN. A standard mixture of 10 ng selected pharmaceuticals/µL mentioned above was prepared by adding 200 µL of each compound and thereafter diluting it to 20 mL MeOH/Millipore water (50:50 v/v). This resulted in a spiking concentration of 52 µg/kg in the dewatered sludge and a total amount of 6.5 µg/soil column (125 g sludge/column). In order to validate the experiment and assure similar transport behaviour from the spiked and non-spiked compounds, a few pharmaceuticals from each group were selected and not spiked in the sewage sludge. The non-spiked pharmaceuticals were SOT (ß-blockers), NFX, RXC (antibiotics), AMT and ZPD (antidepressants). The sludge was spiked according to the procedure from Lapen et al. (2008) and Wu et al. (2010) that used spiking concentrations of 1760 µg/kg and 100 mg/kg sludge, respectively. However, in this study the spiking concentration selected (52 µg/Kg dewatered sludge) was lower in order to investigate the transport of the pharmaceuticals at environmentally relevant concentrations. The homogenized sludge was divided in glass jars of 250 mL (1 jar per soil column and 1 jar for background analysis). To each jar 125 g of homogenized sludge was added. The sludge in each jar was spiked with 650 µL (10 ng/µL) standard mixture. The spiking solution was added in the 250 mL glass jars by adding 15 drops in a star-shaped pattern, mixing the sludge 10 times after each adding. The drops were added in a star shape 3 times and after the third time the sludge was mixed 30 times by mixing from the edges of the glass jars to the middle. The spiking procedure took approximately 4 minutes per sample. The spiked sludge samples were stored in room temperature between January 27, 2015 and February 2, 2015, in order to let the pharmaceuticals adsorb to the sludge.

3.4.2 Leaching experiment

The leaching experiment was initiated February 2, 2015, according to the similar procedure as Jarvis et al. (2008) and Svanbäck et al. (2013). Prior to the start of the experiment, the soils were stored at +18 ˚C for 72 hours which was equivalent to the temperature the experiment was carries out. The experiment was set up by placing 12 soil columns in 12 syphons where a nylon fabric mesh filter of 50 µm was placed in the bottom of each syphon in order to prevent contamination of soil particles in the leachates (Figure 1).

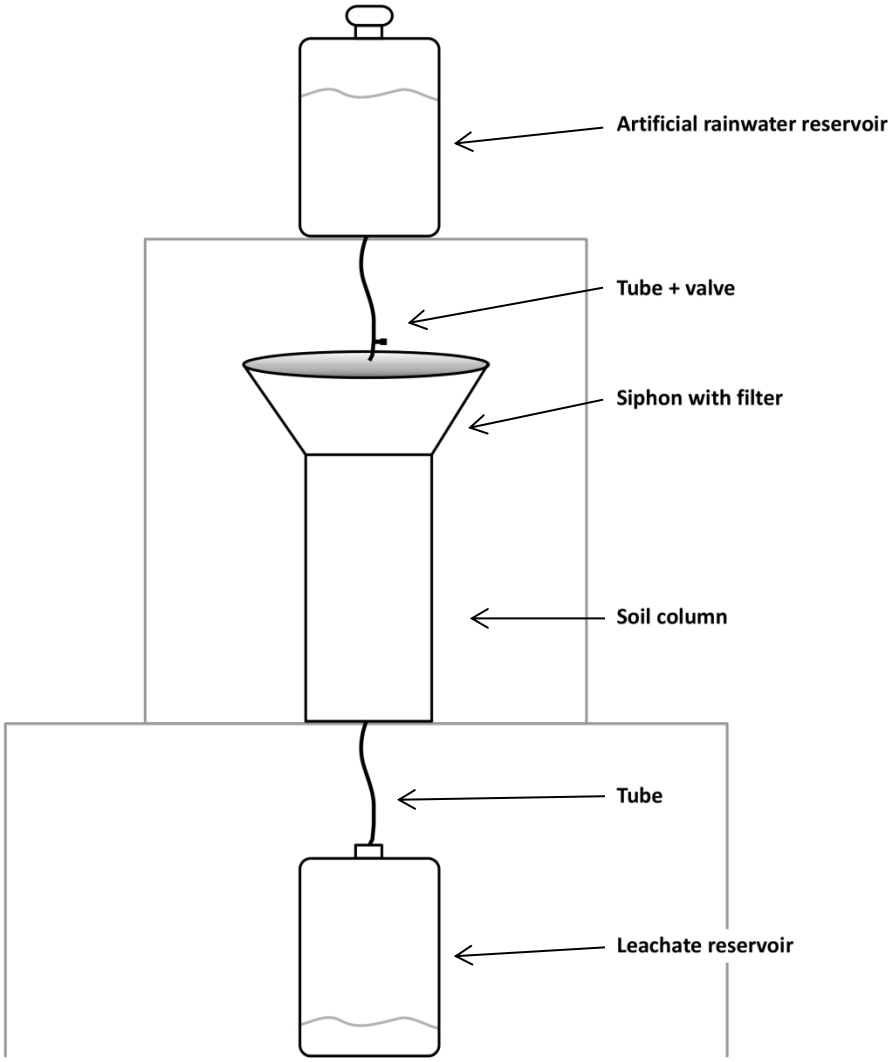


Figure 1. Image of the setup of the leaching experiment.

The columns containing three different soil types; clay, loam and loamy sand were all set up in sets of quadruplicates. The columns were set up in the laboratory with the base of the columns exposed so that free drainage could occur (Jarvis et al. 2008; Larsbo et al. 2009[b]; Larsbo et al. 2013).

In order to mimic the natural release of pharmaceuticals to the soil profile a common sludge application rate was used, as in the study of Lapen et al. (2008) and Wu et al. (2010). The amounts mentioned in previous studies were modified according to annual Swedish sludge application rates of approximately 4 ton de-watered sludge/ha.

To reduce the transport cost of the sludge application a 5 year load is usually applied leading to approximately 20 ton dewatered sludge/ha (Svenskt vatten, 2014). This application rate was used for the experiment giving that approximately 125 g sludge per column was applied. In order to mimic field incorporation of sludge in soil, 5 cm of top soil was excavated from the upper layer of the soil columns and mixed with 125 g spiked sludge. The soil and sludge were mixed in a 2 L glass beaker for approximately 2 minutes, one minute clockwise and one minute counter-clockwise in order to ensure complete and even mixing. The soil and sludge mixture was then returned to the soil column and gently pressed into place (Richards et al. 2000).

The experiment was carried out using a rainfall simulator, described in detail by Ahrens (unpublished). The experiment was carried out over a 34-day period starting on February 2 and ending on March 8, 2015. In order to ensure leaching of the pharmaceuticals over the period of the experiment, a rainfall intensity of 1.6 mm/h was chosen. Therefore, approximately 1 L of artificial rainwater (0.01 M $CaCl_2$ Millipore) was added to each column over a 48 hour interval. From the rainfall simulators, tubes with valves attached were located above the columns. Siphons equipped with PVC sieves covered with a Ø 125 mm filter paper (Munktell, Ahlstrom) were placed on top of the soil columns. This, in order to ensure even distribution of the artificial rainwater (Figure 1).

Over a 48 hour interval approximately 1 L of leachate was collected in 1 L polyethylene bottles from each column (1 L rainfall/48 hours). The pooling scheme can be seen in Table 4 below. Due to analysis purposes, 2 L of leachate from 4 days was pooled. This was done during the first 16 days of the experiment. For the remaining 18 days, 3 L of leachate from 6 days was pooled. This resulted in seven pools in total for each column. In order to normalise the analysis, all leachate volumes were pooled to 1 L, taking 50 % from each leachate for day 4 – 16 and 33 % from each leachate bottle day 22 – 34.

Table 4. Pooling scheme of the volumes taken out from each column (L).

Period (day)	0-4	4-8	8-12	12-16	16-22	22-28	28-34
Approximate leachate volume taken out (L)	2	2	2	2	3	3	3
Approximate final pooled volume analysed (L)	1	1	1	1	1	1	1

3.5 Analysis of pharmaceuticals

3.5.1 Extraction of leachate samples

The extraction of the leachates samples was done by firstly filtering the full volume of the leachate using glass microfiber filter of 0.7 µm. 250 mL of each filtrated leachate sample was transferred to a 250 mL Schott glass bottle and initial pH was measured. To each sample 50 µL (1 ng/µL) pharmaceutical isotopic standard (IS) mixture and 7.5 mL of a 0.1 M Na_2EDTA solution was added and the bottles thoroughly shaken. The glass bottles were thereafter adjusted to pH 3 by dropwise addition of formic acid and thoroughly shaken between the additions in order to ensure even distribution. Prior to the extraction the full volume of the leachate sample was weighted.

The pharmaceutical residues in the leachate were extracted by solid-phase extraction (SPE) using Oasis hydrophilic-lipophilic balance (HLB) cartridges (6cc, 200 mg, 30 µm, Waters). Before loading the leachate samples the cartridges were pre-conditioned with 6 mL MeOH followed by 6 mL of Millipore water acidified with formic acid to a pH of 3. The cartridges were loaded with 250 mL of the filtrated samples. After sample loading, the cartridges were rinsed with 6 mL of Millipore water acidified to pH 3. The cartridges were dried by centrifugation at 3500 rpm for 5 minutes in order to remove remaining excess water. The leachate samples were eluted with 8 mL of MeOH. The eluate was thereafter concentrated until complete dryness using a nitrogen evaporator. Thereafter the solution was evaporated to complete dryness and thereafter stored at -20˚C until analysis. For period 0 - 8, one duplicate was used and for period 8 – 34, two duplicates. Extracts were reconstructed by the addition of 100 µL MeOH and 900 µL Millipore water.

3.5.2 Extraction of sludge samples

By the start of the leaching experiment (2 February, 2015), 125 g of unspiked and 125 g of spiked sludge was frozen in order to compare the initial pharmaceutical concentrations in the sludge and to evaluate the percentage of measured concentration of the compounds in the sludge compared to the spiked. The percentage of the spiked concentrations was calculated by dividing the concentration in the spiked sludge with the initial concentration in the sludge and the spiking concentration. Both sludge samples were freeze dried for 48 hours. Prior to the extraction the samples were grinded in a porcelain mortar in order to achieve homogenous samples. Samples were stored at +4˚C before analysis.

Two different extraction methods were used in order to determine the concentrations of pharmaceuticals in sludge, method A and method B.

Method A was used to analyse AMT, CBZ, FXT, MET and LTG. Method B was used in order to analyse ATL, CIT, DZP, OZP, PRO, TMP and VEN. All sludge samples were analysed in duplicates for both methods.

Method A

In Method A, 2 g homogenized freeze dried sludge was weighted in 50 mL PP tubes. To all samples, 2 mL 100 mM sodium hydroxide solution (NaOH in 80% methanol and 20% Millipore water) was added and samples were let to soak for 30 minutes. Thereafter 20 mL of MeOH and 100 µL (1 ng/µL) pharmaceutical isotopic standard (IS) mixture was added to each sample. The PP-tubes were placed in a wrist-action shaker at 200 rpm for 60 minutes and thereafter centrifuged at 3000 rpm for 15 minutes. Thereafter the supernatant of each sample was decanted in to new 50 mL PP-tubes. The extraction procedure was repeated for the remaining sludge with the exception that only 1 mL 100 mM sodium hydroxide solution (NaOH in 80% methanol and 20% Millipore water) and 10 mL MeOH was added. The PP-tubes were placed in a wrist-action shaker at 200 rpm for 30 minutes and thereafter centrifuged at 3000 rpm for 15 minutes. Thereafter, the remaining supernatant was decanted to the first PP-tube. The decanted sample, 0.1 mL 4 M hydrochloric acid (HCl) was added and the sample shook by hand. Subsequently, the tubes were centrifuged at 3000 rpm for 5 minutes. A volume of 15 mL of the sample extract (half of the sample extract) was transferred to a 15 mL PP-tube and concentrated to 1 mL using a nitrogen evaporator. The samples were frozen at -20˚C for one hour and centrifuged at 3500 rpm for 5 minutes.

Thereafter, the supernatant was decanted to a new HPLC vial. For the clean-up, 25 mg ENVI-Carb and 50 µL glacial acetic acid was added to a 1.7 mL Eppendorf centrifuge tube. The 1 mL samples were added to the Eppendorf centrifuge tubes and vortexted for 30 seconds. Thereafter the samples were centrifuged at 4000 rpm for 15 minutes. The 1 mL samples were transferred to a HPLC vial and evaporated under nitrogen gas to complete dryness. The samples were stored at -20˚C until the time of analysis and prior to analysis they were reconstituted by addition of 300 µL MeOH and 700 µL Millipore water.

Method B

Method B was initiated by weighing 2 g homogenized freeze dried sludge in 50 mL PP tubes. To all samples, 50 µL (1 ng/µL) pharmaceutical isotopic standard (IS) mixture was added. The samples were thereafter vortexed in order to homogenize and mix well. After addition of the isotopic standard mix, the samples were left for 30 minutes before extraction. To each sample, 10 mL of Na_2EDTA (0.1 M) and 10 mL of Acetonitrile (ACN, 1 % acetic acid) was added. After each addition, the samples were vortexed for 30 seconds.

Thereafter, 1.5 g NaOC and 6 g $MgSO_4$ was added, each sample was manually shook for 30 seconds and vortexed for 1 minute. The samples were thereafter centrifuged for 15 minutes at 3500 rpm. After centrifugation, 10 mL of supernatant was decanted from each sample and added to 15 mL PP tube with 900 mg $MgSO_4$ and 150 mg PSA. Each sample was manually shook for 30 seconds, vortexed for 1 minute and thereafter centrifuged for 15 minutes at 3500 rpm. After centrifugation, approximately 9 mL of supernatant was decanted to 10 mL glass tubes. The samples were thereafter concentrated using a nitrogen evaporator until 200 µL of sample was left. The sample was thereafter transferred to a 1 mL HPLC vial by rinsing the glass tubes twice with 200 µL acetonitrile (ACN). The extract was frozen for one hour at -20˚C and thereafter centrifuged for 5 minutes at 3500 rpm. The supernatant was transferred to a 1 mL HPLC vial and concentrated under nitrogen gas to complete dryness thereafter stored at -20˚C until analysis. The reconstruction before HPLC analysis was done by addition of 300 µL MeOH and 700 µL Millipore water.

3.6 Instrumental analysis

All samples were analysed with high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS/MS) Gros (unpublished). A separate mixture of isotopically labeled internal standards, used for internal standard calibration, was prepared in methanol.

3.7 Quality assurance and quality control

In order to avoid contamination of the samples, materials and objects that might contain pharmaceuticals were avoided. In order to assure no contamination of SPE cartridges, two blank samples containing Millipore water acidified with formic acid to pH 3 were used. LOD was set to 3 x σ/S for all the samples and LOQ was selected as the lowest concentration detected on the calibration curve.

3.8 Data evaluation and statistical analysis

In order to assure that the leaching patterns for the different soil types were significant a student t-test on 95 % significance level on total ng leached was performed. A student t-test on 95 % significance of the total input and output volumes was also performed in order to investigate if the irregularities of the flow rate were significant. Both student t-tests were generated by Minitab 16. Spearman correlation coefficients were calculated using R (R Core Team, 2015). From this a spearman correlation matrix was created.

4 Results

4.1 Quality assurance and quality control

Two analytical blanks using Millipore water were analysed. The Millipore blanks were analysed simultaneously with samples conducted after 28 days of the experiment. In the Millipore blanks, only CIT was detected. CIT was detected at 0.0069 µg/L which was below LOQ (0.017 – 0.030 µg/L and thereby put as < LOQ, see Table A1 – A3 in the Appendix.

In order to assure that the correct pharmaceuticals had been detected, LOD was calculated as LOD = 3 x σ/S and LOQ as the lowest concentration detected on the calibration curve. The samples that were below LOD have been graded as not detected (n.d.) in table A1 – A3, Appendix.

4.2 Evaluation of the experimental setup

As seen in Table C1 in the Appendix, an irregular flow rate was applied on the columns due to flaws in the rainfall simulator. The statistical data however show that no significant differences between the soil types can be seen with regards to the total volume applied (p-values > 0.05, t-test. Table C2, Appendix). The clay did however get a higher volume applied after 8 days (2.46 L) and did show high standard deviations (0.16 L). However the total volume that leached showed no significant difference between the soil types, as seen in Table C3 and by the statistics in Table C4, Appendix (p > 0.05, t-test).

One of the clay columns clogged after 4 days of the experiment and was thus removed giving clay, n = 3. Table D2 in Appendix shows the average pH analysed for each leachate sample where a slight trend for a decline can be seen.

4.3 Effects of soil texture on leaching pattern

Of the 22 selected pharmaceuticals 12 leached in detectable concentrations (Figure 2, Figure 3 and Table A1 – A3, Appendix). Of the 12 pharmaceuticals that leached, 5 were found to leach from loamy sand: CBZ, CIT, MET, OZP and VEN (Table A1 – A3, Appendix). From loam, 10 pharmaceuticals leached: ATL, CBZ, CIT, DZP, FXT, MET, OZP, PRO, SRT and VEN (Table A1 – A3, Appendix). From clay, 12 leached: ATL, CBZ, CIT, DZP, FXT, LTG, MET, OZP, PRO, SRT, TMP and VEN (Table A1 – A3, Appendix). Most pharmaceuticals that did not leach belong to the group of antibiotics; AZC, CFX, CLC, NFX, OFX, RXC, SMX. The others that did not leach were SOT (ß-blocker), AMT and ZPD (antidepressant).

All pharmaceuticals that leach in loamy sand apart from CIT show a gradual increase in leaching (MET Figure 2 and CBZ, OZP, VEN Figure 3). For MET and CBZ there is no clear difference in leaching concentrations between clay and loam (Figure 2 and Figure 3). The student t-test gave p-values > 0.05 for the first two leaching points for both compounds (Table C5, Appendix) indicating no significant differences in leaching.

DZP does not leach in loamy sand but in loam and clay (Figure 3) where it in clay leaches with higher concentration, error bars not overlapping. TMP and LTG do not leach in loamy sand or loam but in clay (Figure 3). LTG shows a tendency to stop leaching after 34 days (Figure 3) whereas TMP stops leaching completely after 28 days (Figure 3).

CIT shows similar leaching pattern for all soil textures with peaks after 8 days (Figure 2). FXT does not leach in loamy sand but in loam and clay with a peak after 8 days (Figure 2) showing no difference in leaching pattern. The same can be seen for ATL (Figure 2).

SRT is not seen to leach in loamy sand and has a delayed leaching pattern in both loam and clay (Figure 2). In loam it shows a slight tendency to decline in leaching whereas in clay it shows a tendency to decrease leaching after 16 days.

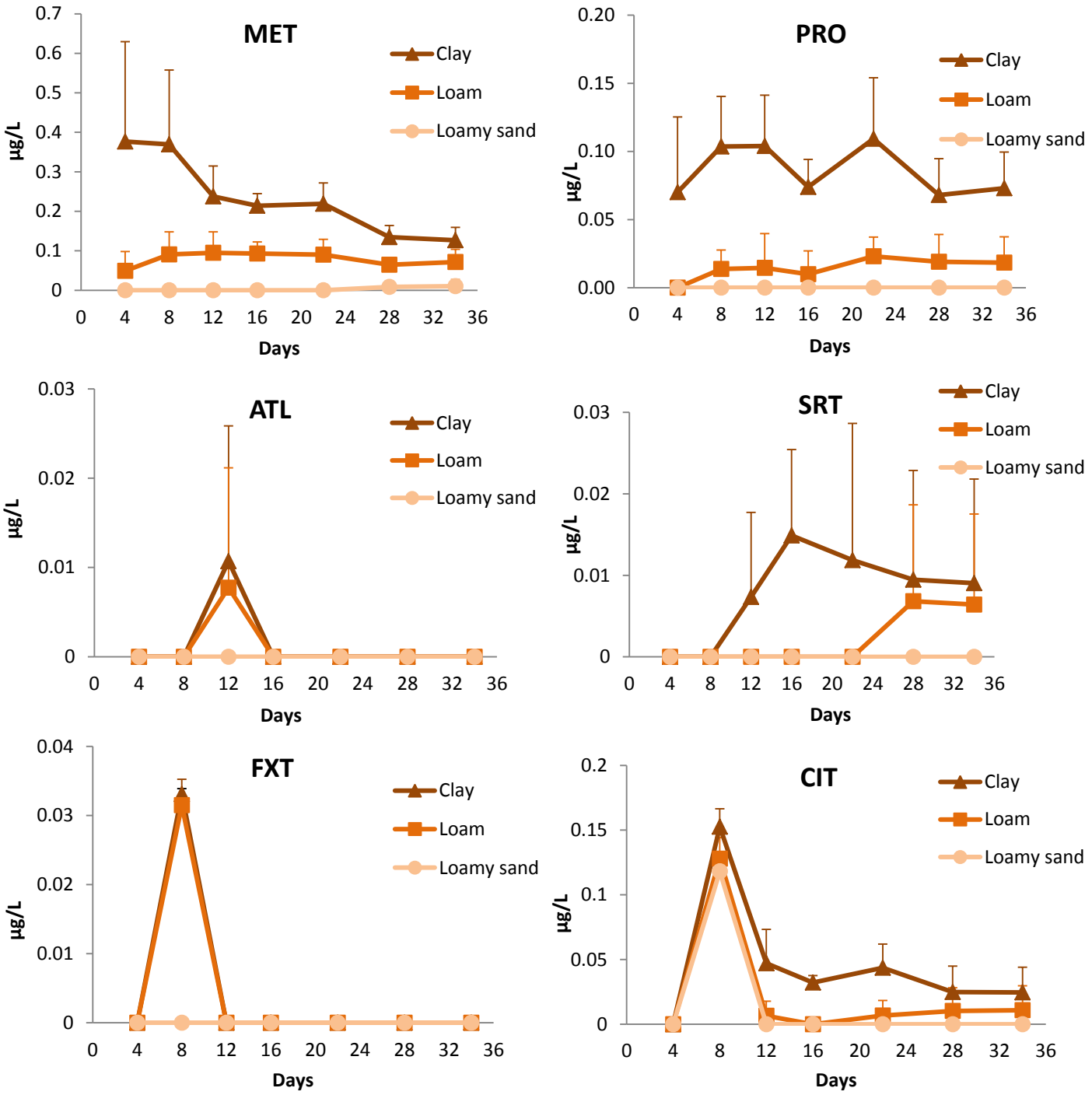


Figure 2. Leaching patterns from different soil types in (µg/L) over days. Scales (µg/L) on the y-ax vary between compounds in order to show a clear leaching pattern. Error bars represent standard deviation (n = 3) for clay and (n = 4) for loam and loamy sand.

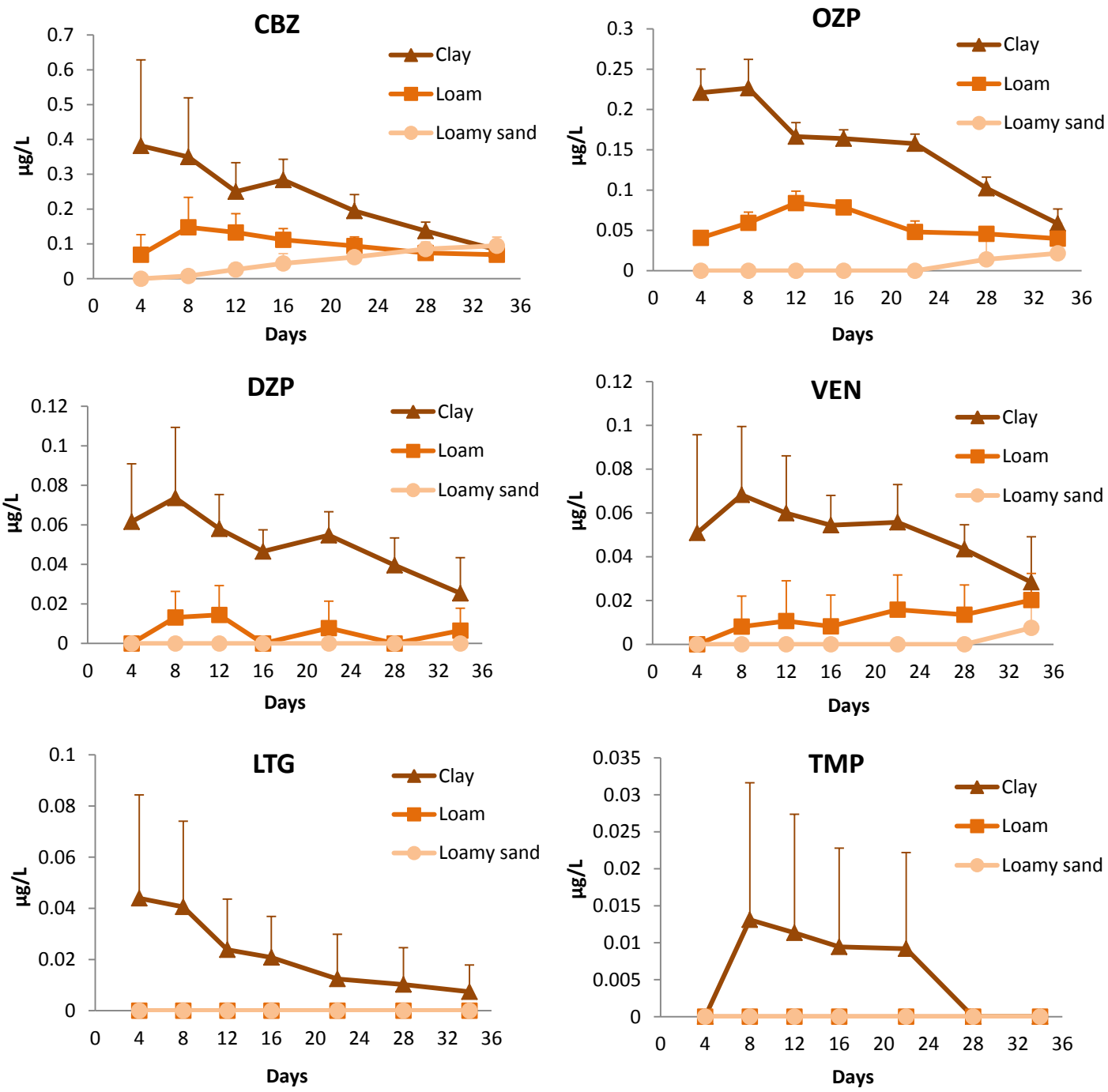


Figure 3. Leaching patterns from different soil types in (µg/L) over days. Scales (µg/L) on the y-ax vary between compounds in order to show a clear leaching pattern. Error bars represent standard deviation (n = 3) for clay and (n = 4) for loam and loamy sand.

4.4 Comparison of leaching pattern

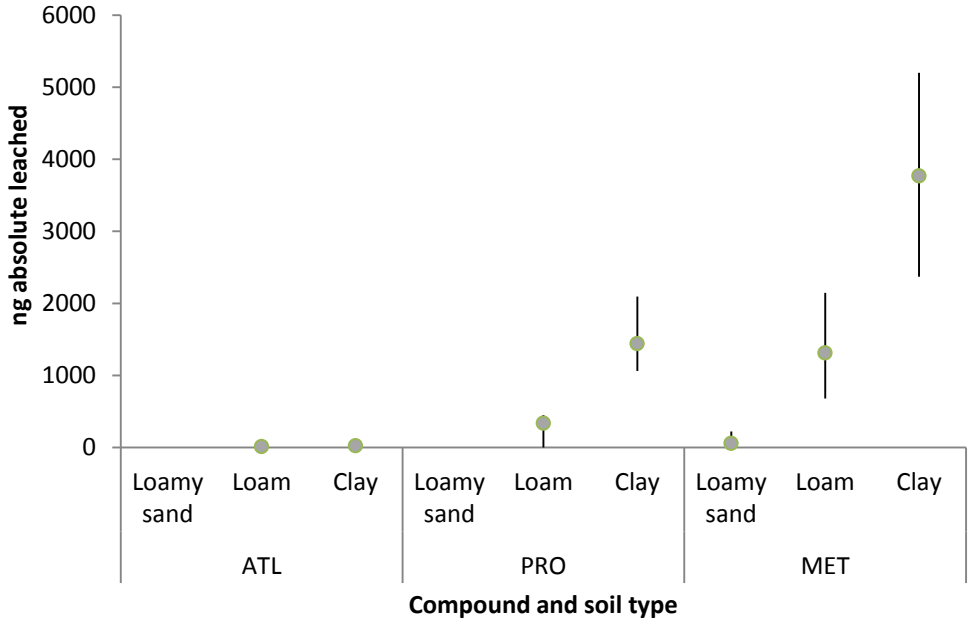


Figure 4. Highest, lowest and average values of (ng) absolute ß-blockers leached per soil column depending on soil texture.

A difference in average mass leached from loam and clay can be seen for both MET and PRO (Figure 4). The leaching of both MET and PRO was significantly higher from clay than loam ($p < 0.05$, t-test, Table 5). For ATL there is no significant difference ($p > 0.05$, t-test, Table 5) between mass leached from loam and clay regarding the mean values of the mass leached (Figure 4).

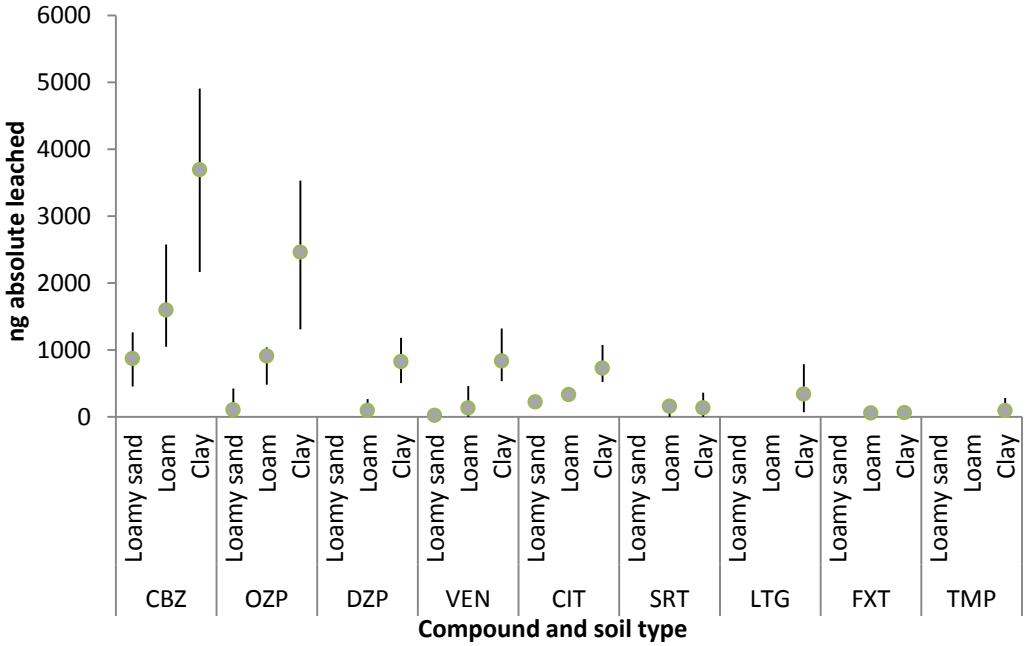


Figure 5. Highest, lowest and average values of (ng) absolute antidepressants and TMP leached depending on soil texture.

For anti-depressants the total leaching was significantly higher from clay than loam for 4 out of 8 substances ($p < 0.05$, t-test, Table 5).

Table 5. P-value derived from student two sided t-test on 95 % significance level in the total ng leached from different soil types, $p < 0.05$ shows significant differences (*)[a].

Compound	loamy sand vs loam	loam vs clay	loamy sand vs clay
ATL	n.d.	0.678	n.d.
PRO	n.d.	0.011*	n.d.
MET	0.010*	0.027*	0.003*
CBZ	0.102	0.044*	0.010*
FXT	n.d.	0.550	n.d.
CIT	0.060	0.157	0.102
DZP	n.d.	0.010*	n.d.
OZP	0.012*	0.046*	0.008*
LTG	n.d.	n.d.	n.d.
SRT	n.d.	0.413	n.d.
VEN	0.148	0.045*	0.011*
TMP	n.d.	n.d.	n.d.

[a]n.d. = not detected.

4.5 Mass balance

For ATL, less than 1 % of the mass contained in the biosolids leached from both loam and clay (Figure 6). For ATL, the percentage measured in the sludge of the spiked concentration in the biosolids was less than 50 % (Table B1, Appendix). For PRO, 1.8 % of the mass contained in the biosolids leached from clay and 0.2 % from loam (Figure 6). The percentage measured in the sludge of the spiked concentration of PRO in the biosolids was 129 % (Table B1, Appendix). Regarding MET, differences in ratio leached can be seen to follow the leaching patter clay > loam > loamy sand with 13 % > 2.4 % > 0.2 % absolute amount leached. The percentage measured in the sludge of the spiked concentration of MET in the biosolids was 176 %. A higher concentration of PRO (366.1 µg/L) than of MET (113.6 µg/L) was found in the biosolids (Table B1, Appendix).

For CBZ differences in ratio leached can be seen to follow the leaching patter clay > loam > loamy sand with 15 % > 6.5 % > 3.5 % relative amount leached (Figure 6). The percentage measured in the sludge of the spiked concentration of CBZ in the biosolids was 157 % (Table B1, Appendix). For OZP more absolute amount leached than for DZP in all soil types (Figure 6). The percentage measured in the sludge of the spiked concentration in the biosolids for both compounds was above 200 % (Table B1, Appendix). For VEN, 2.4 % of the mass contained in the biosolids leached from clay and less than 0.5 % from loam and loamy sand (Figure 7). The percentage measured in the sludge of the spiked concentration in the biosolids of VEN was 141 % (Table B1, Appendix). For CIT and FXT, less than 0.5 % of the mass contained in the biosolids leached where FXT did not leach from loamy and (Figure 7). For TMP, 0.5 % of the mass contained in the biosolids leached from clay (Figure 7). For CIT and TMP, the percentage measured in the sludge of the spiked concentration in the biosolids was above 200 % (Table B1, Appendix). For FXT the percentage measured in the sludge of the spiked concentration in the biosolids was 100 % (Table B1, Appendix). For LTG, less than 2 % of the mass contained in the biosolids leached from clay (Figure 7). AMT was not found in the leachate whereby absolute amount leached could not be measured. The percentage measured in the sludge of the spiked concentration in the biosolids was below 100 % (Table B1, Appendix).

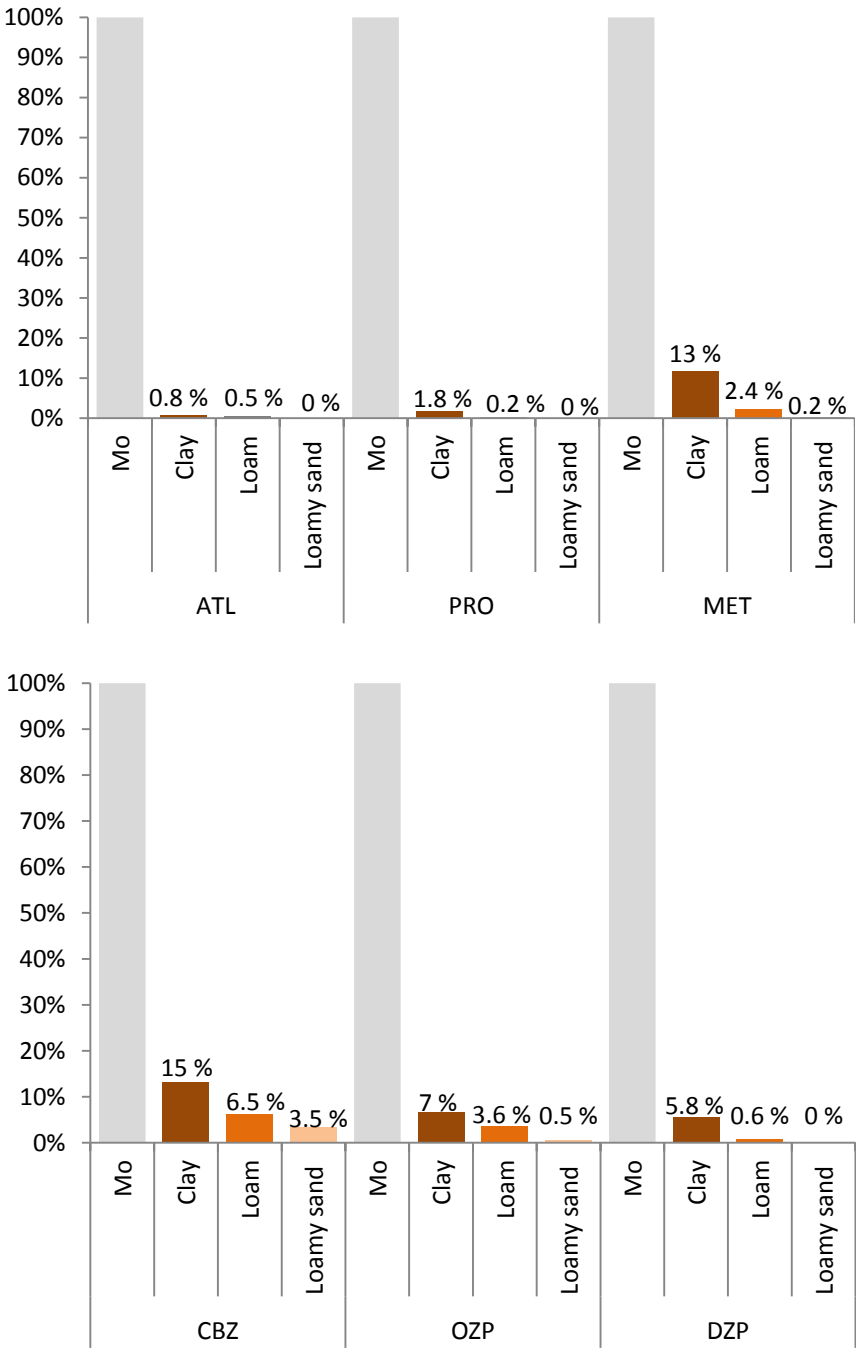


Figure 6. Ratio of initial ng compound analysed in sludge (M_o) after spiking and average ng found in leachate (M) from soil columns with different texture. Each bar representing a fraction (%) of initial ng compound analysed in sludge (M_o) above each bar.

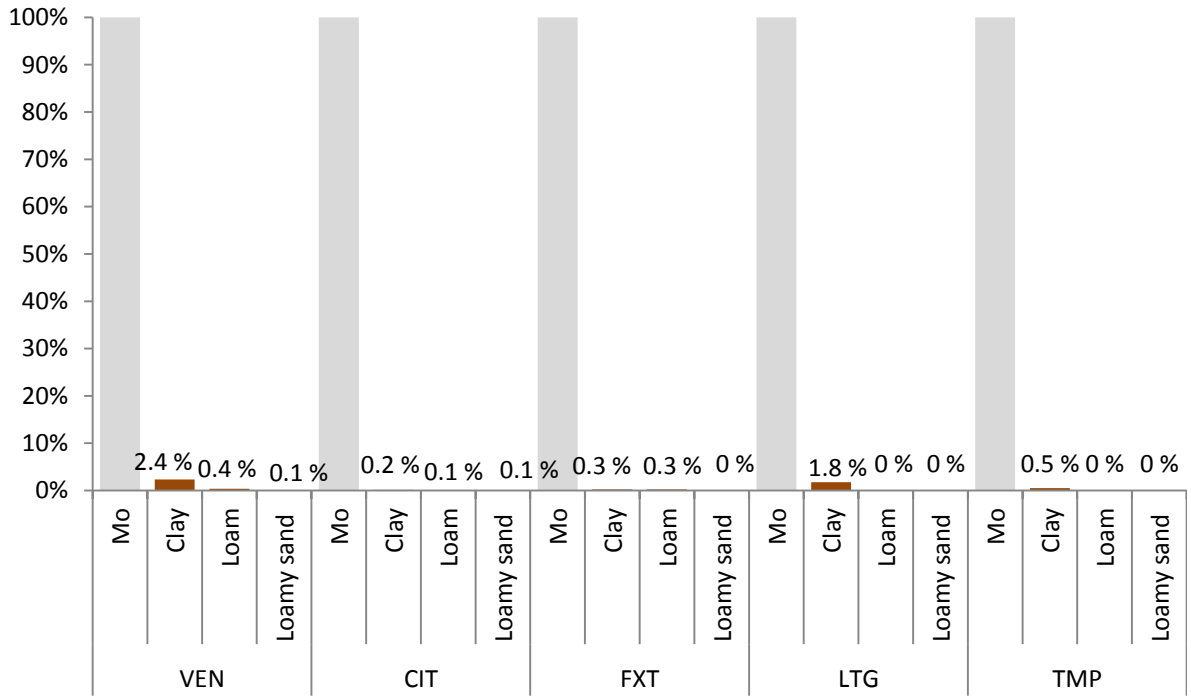


Figure 7. Ratio of initial ng compound analysed in sludge (M_o) after spiking and average ng found in leachate (M) from soil columns with different texture. Each bar representing a fraction (%) of initial ng compound analysed in sludge (M_o) above each bar.

5 Discussion

5.1 Effects of soil texture on leaching pattern.

It is seen that 9 out of 12 compounds leached in the order clay > loam > loamy sand (CBZ, DZP, LTG, MET, OZP, PRO, SRT, TMP and VEN) (Figure 2, Figure 3, Table 5 and Table C5, Appendix). The leaching from clay was higher than the loamy sand despite of the lower organic matter content of the loamy sand (1.24 %) compared to clay (2.03 %) and despite of the lower clay content of the loamy sand (4.30 %) compared to the clay (47.3 %). Previous batch and disturbed column studies have shown adsorption of pharmaceuticals to clay fractions and organic matter (Schaffer et al. 2012; Srinivasan et al. 2013 and Maszkowska et al. 2014). High clay content leads to more adsorption sites compared to sandy soil due to a larger specific surface area of the clay particles, thus, it would be assumed that the pharmaceuticals would be retained to a larger extent in clay compared to loamy sand. However, the higher leaching from clay suggests that the compounds are transported by rapid flow through macropores that can occur in soils with high clay fraction (Eriksson et al. 2005). The low and delayed leaching of 4 of the 5 compounds (CBZ, MET, OZP and VEN) that leached in the loamy sand suggests uniform percolation through mesopores (Eriksson et al. 2005). Since undisturbed soil columns preserve the soil structure and soil macropores (Lewis, J. & Sjöström, J. 2010) it can be assumed that the undisturbed columns of this experiment facilitates transport through the soil structure.

Leaching of pharmaceuticals through macroporous flow has previously been shown by Lapen et al. (2008), Larsbo et al. (2009[a]) and Edwards et al. (2009). Furthermore, pharmaceutical transport through macroporous flow has been shown by D'Alessio et al. (2014) who investigated estrogen transport in undisturbed soil systems.

Since flow in macropores occurs with little or no interaction with the surrounding soil-matrix, the sorption of the compounds to clay and organic matter occurred under non-equilibrium conditions. Macroporous flow in clay soils in the region of Uppsala has been reported by Larsbo et al. (2009[b]), however with regards to herbicide transport. Herbicides and pharmaceuticals can be thought to have similar transport patterns with both groups being ionisable compounds with molecular weights in similar range. Atrazine has been shown to be transported by macroporous flow (Delwiche et al. 2014). With atrazine being a triazine and LTG a phenoltriazine, it is likely that their transport behaviors are similar (Watelle et al. 1997; Delwiche et al. 2014). It can thus be expected that for ionisable compounds such as pharmaceuticals, macroporous flow is the main transport pathway for the 9 compounds that showed increased leaching in clay.

FXT did not show a significant difference in leaching between loam and clay ($p > 0.05$, t-test, Table 5). One explanation might be strong sorption to biosolids which is in agreement with the high log K_{oc} value (5.32) and the study from Gottschall et al. (2012). With the organic carbon content of clay and loam being similar (2.03 % and 2.45 %) and the high log K_{oc} value of FXT (5.32), adsorption to the organic carbon of the soil needs to be considered (Johnson et al. 2005; U.S. EPA. 2012). CIT did not show a significant difference in leaching between the different soil types ($p > 0.05$, t-test, Table 5). This might be explained by that CIT was either sorbed to the sludge due to its high log K_{oc} value (4.40) or retained in the organic carbon of the soil (U.S. EPA. 2012). However, the data for CIT has to be taken with caution since the percentage measured in the sludge was 247 % of the spiked concentration (Table B1, Appendix) which indicate instrumental interferences.

ATL did not show a difference in leaching pattern ($p > 0.05$, t-test, Table 5) with varying soil type in contrast to the other ß-blockers (i.e. MET and PRO) (Figure 2). ATL was found in a lower concentration in the spiked sludge (23.2 µg/kg) than MET (225.5 µg/kg). The low measured concentration of ATL in the sludge compared to the spiked concentration 45 % (Table B1, Appendix) indicate degradation of the compound. This might explain why ATL did not show a difference in leaching pattern.

SRT showed no significant difference in absolute amount leached between the loam and the clay ($p > 0.05$, t-test, Table 5). However, when looking at Figure 2, a difference in leaching pattern can be seen with SRT starting to leach 14 days earlier from clay than loam. The retained pattern shows that there is a suggestion of macroporous flow with faster transport through the clay.

Furthermore the slow leaching of SRT might be a result of the high log D_{ow} value (5.29), indicating retention of the compound as shown by Petrie et al. (2014). Johnson et al. (2005) also showed that SRT strongly sorb to sludge, which was explained by the high log K_{oc} value (5.53).

The compounds SOT and ZPD did not leach which can be explained by the fact that they were not spiked and were not detected in the non-spiked sludge either (Table B1, Appendix). AMT did also not leach but was however detected in both non-spiked and spiked sludge (Table B1, Appendix). The compound has also previously been detected in Swedish sewage sludge (Fick et al. 2011). With the similar structure of AMT and CBZ it could be assumed that AMT leach in a similar way, however it does not. This can be explained by the high log K_{oc} value of AMT (5.70) which suggest that it is strongly bound to either biosolids or the organic carbon of the soil (U.S. EPA. 2012).

In general, the same volumes of artificial rainwater were added to the different soil columns. However, the irrigation rate of 1.6 mm/day was not always maintained on a daily basis (Table C1, Appendix). Statistical analysis showed no significant differences between total volume added and total volume leached ($p > 0.05$, t-test, Table C2 and Table C4, Appendix). Thereby it can be thought that the inconsistent irrigation rate did not affect the leaching patterns of the compounds.

5.2 Correlation of physiochemical properties

A correlation matrix was done correlating the physiochemical properties of the pharmaceuticals and their leaching behavior. Low correlation was found when including all pharmaceuticals investigated (Figure E1, Appendix). No correlation could be performed for the pharmaceutical groups ß-blockers and antibiotics since only 3 and 1 compound, respectively, were detected in the leachate. Thus a correlation was only performed for antidepressants (Figure 8).

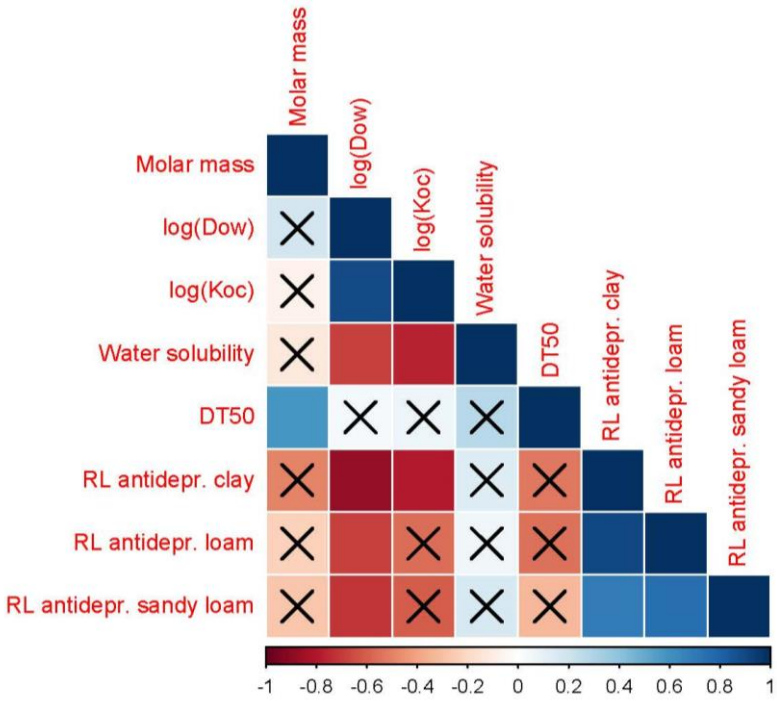


Figure 8. Spearman correlation matrix showing correlation between physiochemical properties of antidepressants and leaching behavior. -1 stands for negative correlation whilst 1 stands for positive. Positive correlation indicates that variables on the y-axis increase when the variables on the x-axis increase. Negative correlation indicates that variables on the y-axis decrease when the variables on the x-axis increase. Blank boxes indicate significant correlation between the y and x variables.

Antidepressants

For the antidepressants, the leaching amount of individual compounds was generally higher for clay compared to loam and loamy sand and decreased as follows: CBZ > OZP > DZP > VEN > LTG (Figure 6 and Figure 7). Log D_{ow} can be seen to be significantly negatively correlated with the relative leaching, with leaching decreasing when log D_{ow} is increasing (Figure 8). Decreased leaching due to high log D_{ow} values has previously been shown by Petrie et al. (2014). The relative mass of CBZ, OZP and DZP leached from clay ranged between 5 % and 15 % compared to the total concentration in the sludge (Figure 6 and Figure 7). The peak of the leaching was reached at day 4 (CBZ) and day 8 (OZP and DZP) and decreased continuously until day 34 which indicate that that the remaining 95% to 85 % is either retained in the soil, sludge or that it is degraded. The pharmaceuticals with a high DT_{50} (i.e. FXT, LTG and VEN) are probably adsorbed to the soil or sludge and the ones with low DT_{50} (i.e. CBZ, OZP and DZP) might have degraded.

De Wilde et al. (2009) showed high DT_{50} values for pesticides to indicate strong sorption to soil due to reduced bioavailability. Thus, the lack of correlation of DT_{50} values with the significant correlation of log D_{ow} (Figure 8) suggests the remaining pharmaceuticals to be adsorbed to the soil matrix. This can also be believed with the leachate pH (pH 3.6 ± 0.2, Table D2, Appendix) being below the pK_a for CBZ, CIT, FXT, LTG, OZP, SRT and VEN (Table 1) giving that they are cationic and would thus bind to the permanent negatively charged clay surfaces (Maszkowska et al. 2014).

Figure 8 show significant negative correlation for log K_{oc} and leaching ($p < 0.05$, spearman correlation) where leaching decreases with increased log K_{oc} in leaching from clay. This is to be expected since pharmaceuticals are known to sorb to organic carbon (Cunningham, V. & Kümmerer, K. 2008). From this study it can not be determined if the pharmaceuticals are sorbed to the organic carbon in the sludge or the soil. However, if investigating the organic fraction of the sludge (8.6 %, Table 3) and clay (2.23 %, Table 2) an indication is given that the pharmaceuticals might be sorbed stronger to sludge compared to clay. However, further research is needed since the pH of the biosolids also affect sorption (Cunningham, V. & Kümmerer, K. 2008) and the pH of the leachate (pH 3.6 ± 0.2, Table D2, Appendix) and sludge (pH 7.5, Table 3) differ. No significant correlation can be seen for log K_{oc} and leaching from loam and loamy sand ($p > 0.05$, spearman correlation). This is believed to be due to too few data points ($n = 6$ and $n = 4$ respectively). Figure 8 shows no significant correlation between S_w and leaching ($p > 0.05$, spearman correlation) which ranged between 17.7 mg/L and 267 mg/L.

No significant correlation can be seen in Figure 8 between M_w and leaching ($p > 0.05$, spearman correlation). This is to be expected with the compounds having molar weights in the similar range (236 – 324 g/mole). No significant correlation can be seen between DT_{50} and leaching ($p > 0.05$, spearman correlation). This might be explained by that the DT_{50} values are high (i.e. 54 – 360 days) compared to the duration of the study, 34 days. Thus, the compounds might not have had time to degrade and thereby correlations cannot be seen. No difference in correlation between leaching between log D_{ow} and log P_{ow} could be seen (Table D1, Appendix). This can be seen as an indication that when looking at leaching behavior of pharmaceuticals in biosolids both values can be used.

ß-blockers

Due to lack of data points (n = 3), no correlation matrix could be made for ß-blockers. However, from Figure 4 and 6 it can be seen that the ß-blockers leached in the magnitude of MET > PRO > ATL. The larger leaching of MET than PRO can be explained by the log D_{ow} value of the compounds, MET having a lower log D_{ow} value (1.92) than PRO (3.48). As shown by Petrie et al. (2014), compounds with a low log D_{ow} have a high tendency to leach. This suggests that log D_{ow} is a relevant indicator to use when correlating leaching behavior of ß-blockers. MET also has a higher S_w value (16 900 mg/L) than PRO (61.7 mg/L) and also leached to a larger extent than PRO. According to U.S. EPA. (2012) and Petrie et al. (2014) compounds with a S_w value between 1000 and 10 000 mg/L have a high leaching potential. This indicates that solubility might also be a parameter relevant to take in to account when investigating leaching.

The lower relative amount leached of ATL compared to MET could be due to degradation. However, the DT_{50} values of the compounds are both 75 days indicating that they would degrade equally as fast. These DT_{50} values are however modelled (ChemSpider, EPISuite (PCKOCWIN v1.66), 2015) and when looking looking at data derived under natural conditions differences can be seen. Ramil et al. (2009) found PRO, MET and ATL to degrade in water/sediment in the following magnitude of 33 > 24 > 3 days. These values cannot be used to estimate the degradation in soil but gives an indication of the magnitude and variation between the degradation of ATL and MET. It can thereby be thought that DT_{50} values derived under natural conditions are important factors to consider when investigating leaching of ß-blockers.

Antibiotics

With only TMP leaching, no correlation matrix could be made for antibiotics (Figure 8). TMP is the only compound from the group diaminopyrimidine which makes it difficult to compare its leaching behavior with the other investigated compounds. TMP is a zwitterionic compound as ZMX and the FQs, where ZMX and FQs have been shown to be removed from sludge to a lesser degree than TMP (Lindberg et al. 2006). This indicates that TMP has a lower tendency to sorb to sludge and thus has a higher leaching potential.

The FQs did not leach and were not found in neither the spiked or non-spiked sludge (Table A1 – A3 and Table B1, Appendix). Although, CFX, OFX and NFX have low log P_{ow} of 0.28, -0.39 and 0.46, respectively, which suggest high leaching potential (Petrie et al. 2014).

The high DT_{50} values of the compounds (i.e. 120, 198 and 189 days) suggest that they have limited bioavailability and thereby predicted not to leach (De Wilde et al. 2009). Golet et al. (2003), Lindberg et al. (2006), Lindberg et al. (2007) and Gottschall et al. (2012) have showed a strong sorption of FQs to biosolids and the limited mobility of the compounds. This suggests that the FQs of this experiment are most likely to be adsorbed to the sludge, despite their low log K_{oc} values (1.55 and 1.65, 1.97 for CFX, OFX and NFX respectively) (U.S. EPA, 2012). However, further research is needed since pH of the biosolids might also affect the sorption of these compounds (Cunningham, V. & Kümmerer, K. 2008) and the pH of the leachate and sludge differ (Table D2, Appendix and Table 3).

The macrolides AZC, CLC and RXC did not leach (Table A1 – A3 and Table B1, Appendix) and were also not detected in the non-spiked biosolids (Table B1, Appendix) despite that they have been detected in biosolids in Sweden (Fick et al. 2011). The log P_{ow} values of AZC (4.02) and RXC (3.16) indicate that they show tendency to leach. RXC has a log P_{ow} of 2.75 and should according to Petrie et al. (2014) leach but it did not in this study. The K_{oc} values for these compound could not be found, however, their high molecular weights (747.95, 748.98 and 873.05 g/mole) indicate that they would sorb strongly (Pal et al. 2010). The ability for macrolides to sorb to biosolids has been confirmed by Hollis, J. (1991) who classified the compounds as immobile. Thus molecular weight might be a good correlation factor for leaching of macrolides with less leaching due to higher molecular weight.

6 Conclusions and future perspectives

The main conclusion of this study is that soil texture can be seen to affect leaching pattern and concentrations of pharmaceuticals from biosolids amended soils at environmental relevant concentrations. This study also shows that log D_{ow} is significantly negatively correlated with leaching of pharmaceuticals ($p < 0.05$, spearman correlation). This can be explained by the fact that pharmaceuticals with a high log D_{ow} are potentially stronger sorbed to soil. This indicates that log D_{ow} is a relevant factor to consider when predicting leaching of pharmaceuticals. The study also showed the need of considering other physicochemical properties such as log K_{oc}, DT_{50} and S_w when predicting the risk of leaching of pharmaceuticals. The study showed no difference in correlating leaching with log D_{ow} or log P_{ow}. By this it can be concluded that when predicting leaching of pharmaceuticals from biosolids both log D_{ow} and log P_{ow} can be used.

The results of this study shows higher leaching of pharmaceuticals in clay compared to loam and loamy sand using undisturbed soil columns investigating the top soil layer. In order to fully understand the leaching behavior of pharmaceuticals the whole soil profile should be investigated, for example by large field-scale lysimeter studies. Usage of field-scale lysimeters would better predict actual field conditions by taking into account natural parameters such as temperature, rainfall but also change in biological activity and pH along the depth of the profile. These factors influence degradation and sorption of pharmaceuticals and should be investigated in order to better understand the transport of the compounds. Furthermore, the width of lysimeters would enable investigation of natural sized macropores.
Natural leaching processes such as surface runoff that occur in the field needs to be investigated in the future.

Ultimately, the results and future perspectives of this study show that there is a need to revise the current legislation for biosolids application on arable land.

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Appendix A

Table A1. Average leaching concentrations (µg/L) in loamy sand with standard deviations (±). N.d. stands for not detected and (0.017 – 0.03 µg/L) < LOQ are concentrations below the lowest concentration detected on the calibration curve. For clay (n = 3), loam (n = 4) and loamy clay (n = 4).

Days	4	8	12	16	22	28	34
ATL	n.d	n.d	n.d	< LOQ	< LOQ	< LOQ	n.d
AMT	n.d	n.d	n.d	n.d	n.d	n.d	n.d
PRO	n.d	n.d	< LOQ	n.d		n.d	n.d
MET	n.d	n.d	< LOQ	< LOQ	< LOQ	0.0085 ± 0.015	0.0103 ± 0.022
SOT	n.d	n.d	n.d	n.d	n.d	n.d	n.d
CBZ	< LOQ	0.008 ± 0.014	0.0263 ± 0.017	0.0438 ± 0.028	0.0620 ± 0.023	0.0851 ± 0.021	0.0947 ± 0.025
FXT	n.d	n.d	n.d	n.d	n.d	n.d	n.d
CIT	n.d	0.1182 ± 0.008	n.d	< LOQ	n.d	n.d	n.d
DZP	n.d	n.d	n.d	n.d	n.d	< LOQ	< LOQ
OZP	n.d	n.d	n.d	n.d	n.d	0.0141 ± 0.024	0.0215 ± 0.037
LTG	n.d	n.d	n.d	n.d	n.d	n.d	< LOQ
SRT	n.d	n.d	n.d	n.d	n.d	n.d	n.d
VEN	n.d	n.d	< LOQ	n.d	< LOQ	< LOQ	0.0075 ± 0.013
ZPD	n.d	n.d	n.d	n.d	n.d	n.d	n.d
TPM	n.d	n.d	n.d	n.d	< LOQ	n.d	n.d
AZC	n.d	n.d	n.d	n.d	n.d	n.d	n.d
CFX	n.d	n.d	n.d	n.d	n.d	n.d	n.d
CLC	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NFX	n.d	n.d	n.d	n.d	n.d	n.d	n.d
OFX	n.d	n.d	n.d	n.d	n.d	n.d	n.d
RXC	n.d	n.d	n.d	n.d	n.d	n.d	n.d
SMX	n.d	n.d	n.d	n.d	n.d	n.d	n.d

Table A2. Average leaching concentrations (µg/L) in loam with standard deviations (±).N.d. stands for not detected and (0.017 – 0.03 µg/L) < LOQ are concentrations below the lowest concentration detected on the calibration curve. For clay (n = 3), loam (n = 4) and loamy clay (n = 4).

Days	4	8	12	16	22	28	34
ATL	n.d	n.d	0.0077 ± 0.0134	n.d	n.d	n.d	< LOQ
AMT	n.d	n.d	n.d	n.d	n.d	n.d	n.d
PRO	< LOQ	0.01371± 0.0139	0.01453 ± 0.025	0.00989 ± 0.017	0.0231 ± 0.039	0.01906 ± 0.019	0.01842 ± 0.018
MET	0.04906 ± 0.049	0.09075 ± 0.057	0.09479 ± 0.053	0.09317 ± 0.029	0.0900 ± 0.039	0.0645 ± 0.016	0.0715 ± 0.032
SOT	n.d	n.d	n.d	n.d	n.d	n.d	n.d
CBZ	0.0691 ± 0.057	0.1478 ± 0.085	0.1327 ± 0.054	0.1116 ± 0.032	0.0938 ± 0.026	0.0740 ± 0.020	0.0688 ± 0.021
FXT	n.d	0.03150 ±0.003	n.d	n.d	n.d	n.d	n.d
CIT	n.d	0.1279 ± 0.016	0.0065 ± 0.011	< LOQ	0.00674 ± 0.011	0.0103± 0.018	0.0109 ± 0.019
DZP	< LOQ	0.0131 ±0.013	0.0145 ± 0.015	< LOQ	0.0078 ± 0.014	< LOQ	0.0065 ± 0.011
OZP	0.0407 ± 0.041	0.0594 ± 0.059	0.0839 ± 0.03	0.0785 ± 0.019	0.04806 ± 0.028	0.0458 ± 0.005	0.0398 ± 0.024
LTG	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
SRT	n.d	n.d	n.d	n.d	n.d	0.00683 ± 0.012	0.0064 ± 0.011
VEN	n.d	0.00807 ± 0.014	0.01062 ± 0.018	0.0082 ± 0.014	0.01582 ± 0.015	0.0135 ± 0.0135	0.0202 ± 0.012
ZPD	n.d	n.d	n.d	n.d	n.d	n.d	n.d
TPM	n.d	n.d	n.d	n.d	< LOQ	< LOQ	< LOQ
AZC	n.d	n.d	n.d	n.d	n.d	n.d	n.d
CFX	n.d	n.d	n.d	n.d	n.d	n.d	n.d
CLC	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NFX	n.d	n.d	n.d	n.d	n.d	n.d	n.d
OFX	n.d	n.d	n.d	n.d	n.d	n.d	n.d
RXC	n.d	n.d	n.d	n.d	n.d	n.d	n.d
SMX	n.d	n.d	n.d	n.d	n.d	n.d	n.d

Table A3. Average leaching concentrations (µg/L) in clay with standard deviations (±).N.d. stands for not detected and (0.017 – 0.03 µg/L) < LOQ are concentrations below the lowest concentration detected on the calibration curve. For clay (n = 3), loam (n = 4) and loamy clay (n=4).

Days	4	8	12	16	22	28	34
ATL	n.d	n.d	0.0107 ± 0.015	< LOQ	< LOQ	n.d	n.d
AMT	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	0.0701 ±	0.1036 ±	0.1039 ±	0.0739 ±	0.1093 ±	0.0680 ±	0.0730 ±
PRO	0.055	0.037	0.037	0.020	0.045	0.027	0.026
	0.3767 ±	0.3693 ±	0.2378 ±	0.2141 ±	0.2191 ±	0.1345 ±	0.1268 ±
MET	0.252	0.188	0.077	0.031	0.052	0.02957	0.032
SOT	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	0.3817 ±	0.3499 ±	0.2502 ±	0.2837 ±	0.1945 ±	0.1370 ±	0.0830 ±
CBZ	0.247	0.169	0.083	0.059	0.047	0.025	0.022
FXT	n.d	0.0331 ± 0.001	n.d	n.d	n.d	n.d	n.d
		0.1525 ±	0.0471 ±	0.0322 ±	0.0436 ±	0.0248 ±	0.0245 ±
CIT	n.d	0.014	0.026	0.005	0.018	0.020	0.019
	0.0616 ±	0.0736 ±	0.0580 ±	0.0465 ±	0.0547 ±	0.0396 ±	0.0253 ±
DZP	0.029	0.036	0.017	0.011	0.012	0.014	0.018
	0.2208 ±	0.2265 ±	0.1664 ±	0.1638 ±	0.1576 ±	0.1024 ±	0.0584 ±
OZP	0.128	0.118	0.046	0.046	0.051	0.032	0.041
	0.0439 ±	0.0405 ±	0.0237 ±	0.0208 ±	0.01237 ±	0.0102 ±	0.0074±
LTG	0.040	0.033	0.019	0.016	0.017	0.014	0.010
SRT	n.d	n.d	0.0073 ± 0.010	0.0148 ± 0.010	0.0118 ± 0.017	0.0094 ± 0.013	0.0090 ± 0.013
	0.05089 ±	0.0682 ±	0.0599 ±	0.0544 ±	0.0557 ±	0.0434 ±	0.0283 ±
VEN	0.045	0.031	0.026	0.013	0.017	0.011	0.021
ZPD	n.d	n.d	n.d	n.d	n.d	n.d	n.d
TPM	n.d	0.0131 ± 0.018	0.0113± 0.016	0.0094 ± 0.01334	0.0091 ± 0.0130	< LOQ	< LOQ
AZC	n.d	n.d	n.d	n.d	n.d	n.d	n.d
CFX	n.d	n.d	n.d	n.d	n.d	n.d	n.d
CLC	n.d	n.d	n.d	n.d	n.d	n.d	n.d
NFX	n.d	n.d	n.d	n.d	n.d	n.d	n.d
OFX	n.d	n.d	n.d	n.d	n.d	n.d	n.d
RXC	n.d	n.d	n.d	n.d	n.d	n.d	n.d
SMX	n.d	n.d	n.d	n.d	n.d	n.d	n.d

Appendix B

Table B1. Concentration (µg/kg) in biosolids prior spiking and post-spiking. Percentage of spiked concentration (52 µg/kg) calculated on the basis of the spiking concentration.

Compound	Concentration prior spiking	Concentration post-spiking	% of spiked
ATL	n.d.	23.2	45
MET	76.5	225.5	176
PRO	231.2	366.1	129
SOT	n.d.	n.d.	
CBZ	73.3	196.0	157
CIT	1623.4	4136	247
DZP	n.d.	113.6	218
OZP	79.45	366.1	279
LTG	28.8	153.2	189
SRT	n.d.	n.d.	
VEN	145.9	280.5	141
TMP	n.d.	143.9	277
FXT	85.7	199.9	100
AMT	90.2	110.3	80
ZPD	n.d	n.d	
AZC	n.d.	n.d.	
CFX	n.d.	n.d.	
CLC	n.d.	n.d.	
NFX	n.d.	n.d.	
OFX	n.d.	n.d.	
RXC	n.d.	n.d.	
SMX	n.d.	n.d.	

Appendix C

Table C1. Average input volumes (L) for each day with standard deviations (±), for clay (n = 3), loam (n = 4) and loamy clay (n=4).

	4	8	12	16	22	28	34
Loamy sand	2.52 ± 0.03	2.01 ± 0.01	1.96 ± 0.11	2.47 ± 0.11	2.99 ± 0.02	3.02 ± 0.06	3.06 ± 0.01
Loam	2.52 ± 0.15	2.002 ± 0.01	1.98 ± 0.01	2.43 ± 0.11	2.98 ± 0.04	3.02 ± 0.01	3.06 ± 0.01
Clay	2.55 ± 0.11	2.46 ± 0.16	1.79 ± 0.11	2.34 ± 0.26	3.03 ± 0.02	3.03 ± 0.01	3.06 ± 0.01

Table C2. Results from student t-test on 95 % significance level on the total input volumes (L) for each soil type. $p < 0.05$ shows significant differences.

Soil type	p-value
loamy sand vs loam	0.58
loamy sand vs clay	0.13
loam vs clay	0.08

Table C3. Average output volumes (L) for each day with standard deviations (±), for clay (n = 3), loam (n = 4) and loamy clay (n=4).

	4	8	12	16	22	28	34
Loamy sand	1.85 ± 0.12	1.88 ± 0.14	1.88 ± 0.18	1.96 ± 0.10	3.02 ± 0.10	2.94 ± 0.02	2.99 ± 0.03
Loam	1.99 ± 0.18	1.84 ± 0.16	1.83 ± 0.20	2.06 ± 0.07	2.97 ± 0.10	2.96 ± 0.10	2.99 ± 0.01
Clay	1.95 ± 0.05	1.90 ± 0.04	2.11 ± 0.05	1.83 ± 0.09	3.01 ± 0.11	2.94 ± 0.01	3.00 ± 0.02

Table C4. Results from student t-test on 95 % significance level on the total input volumes (L) for each soil type. $p < 0.05$ shows significant differences.

Soil type	p-value
loamy sand vs loam	0.259
loamy sand vs clay	0.129
loam vs clay	0.184

Table C5. Two sided t-test on 95 % significance level performed on the first two leaching points between clay and loam. $p < 0.05$ shows significant differences.

	p-value - Sample point 1	p-value - Sample point 2
MET	0.106	0.090
CBZ	0.110	0.130

Appendix D

Table D1. pH-correlated log P_{ow} value, log D_{ow}. Derived from Equation 1 and pK_a-values from Table 1 using the average pH value of the leachate (3.6 ± 0.2). Log D_{ow} values were not calculated for zwitterionic compounds.

Compound	Log P_{ow}	Log D_{ow}
ATL	0.16	0.16
MET	1.92	1.92
PRO	3.48	3.48
SOT	0.24	0.24
AMT	4.92	4.92
CBZ	2.45	2.45
CIT	3.74	3.74
DZP	2.82	3.43
FXT	4.05	4.05
LTG	2.57	2.58
OZP	2.24	2.24
SRT	5.29	5.29
VEN	3.28	3.28
ZPD	3.85	3.85
AZC	4.02	4.02
CFX	0.28	0.28
RXC	2.75	2.75

Table D2. Average leachate pH for each soil type over the time span of the experiment with standard deviations (±), for clay (n = 3), loam (n = 4) and loamy clay (n=4).

Days	4	8	12	16	22	28	34
Loamy sand	3.8 ± 0.04	3.6 ± 0.01	3.5 ± 0.03	3.5 ± 0.05	3.5 ± 0.02	3.6 ± 0.02	3.5 ± 0.02
Loam	3.8 ± 0.02	3.7 ± 0.08	3.8 ± 0.01	3.5 ± 0.02	3.6 ± 0.02	3.6 ± 0.05	3.5 ± 0.04
Clay	3.9 ± 0.01	3.7 ± 0.05	3.6 ± 0.10	3.5 ± 0.05	3.5 ± 0.03	3.5 ± 0.01	3.6 ± 0.02

Appendix E

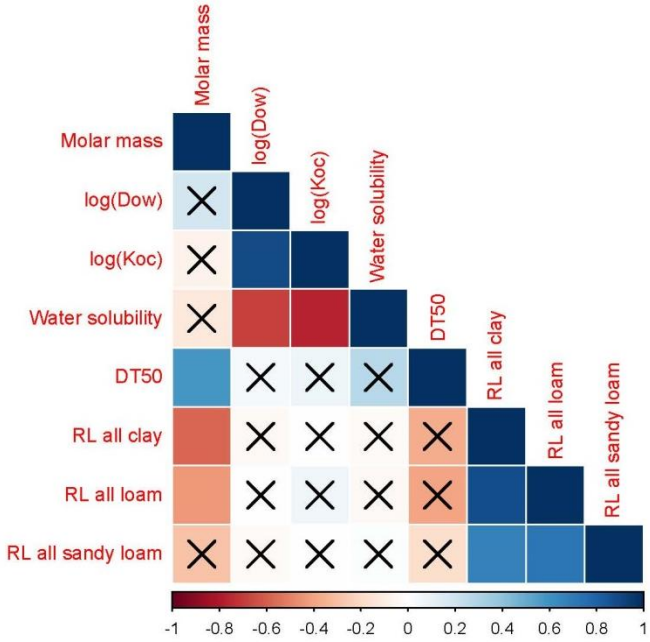

Figure E1.Spearman correlation matrix showing correlation between physiochemical properties of all compounds and their leaching behavior. -1 stands for negative correlation whilst 1 stands for positive. Positive correlation indicates that variables on the y-axis increase when the variables on the x-axis increase. Negative correlation indicates that variables on the y-axis decrease when the variables on the x-axis increase. Blank boxes indicate significant correlation between the y and x variables.