Effectiveness and Utility of Surface Application and Soil Percolation for Removal of Pharmaceutical and Personal Care Products

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Executive Summary

Pharmaceuticals and Personal Care Products (PPCPs) are used worldwide and enter the environment via wastewater treatment system effluent discharges. With increasing concern in water reuse, attention is turning to the wastewater resulting in detection of these compounds in several environmental media associated with wastewater stream. One of the most common reuse procedures is land application of wastewater, which follows a wastewater treatment plant or an onsite wastewater treatment system (OWTS). It is not known how well conventional on-site wastewater disposal practices function in removing PPCPs prior to percolation into ground water. The objective of this study is to investigate the removal of PPCPs from onsite wastewater treatment systems where the final discharge is on the soil surface and to compare the capacity of three land application structures, vegetated lawn, bare land and unvegetated land covered from the sun, in pharmaceuticals removal. In other words, the effect of grass and photodegradation in removal of the pharmaceuticals in land applied systems were assessed. To answer the objectives, three different types of soil columns: grass-covered, plastic-covered, and open columns, each with three replications, were irrigated daily with synthetic wastewater. Leachate samples were collected monthly and analyzed for pharmaceuticals concentrations using HPLC-MS analytical devices.

The removal efficiencies were more than 95% for all compounds in all systems. Pharmaceuticals concentrations in leachates of different column types were not significantly different; so it is concluded that the effect of grass and photodegradation is minimal in the removal process of these compounds in the soil systems. However from the stand point of mass removed in each column type, the statistical analysis shows that grassed columns have been 30% and 15 % more capable of pharmaceuticals removal rather than covered and open columns, respectively which is basically because of higher application rate due to grass maintenance. This means even though vegetated land needs more water to maintain the grass; the system is capable of removing the pharmaceuticals present in the wastewater effluent. Since leachate concentrations of target compounds were less than 5% of the applied amount, it was hypothesized that the pharmaceuticals are mainly accumulated in soil and plant tissue. Therefore, plant and soil samples from different depths of the columns were collected to determine the accumulated mass for mass balance analysis. Soil and plant samples were analyzed using HPLC-UV and LC-MS/MS methodologies. The accumulated amounts for the fluoxetine and acetaminophen were below the detection limits for all samples while carbamazepine and trimethoprim were detected in every soil and plant samples. Carbamazepine was detected at higher concentrations in the soil and plant samples comparing to trimethoprim while the calculated plant bioconcentration factor for trimethoprim was higher than carbamazepine. The results indicate that the mean accumulated amount in the grass samples are significantly ($p<0.05$) higher than the mean accumulated mass in the soil samples. The total mass accumulated in soil and plant samples was less than 1% of the applied mass to the columns suggesting that possible biotransformation processes are involved.

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Introduction

Pharmaceuticals and personal care products (PPCPs) are compounds used worldwide by individuals for health care, as over the counter (OTC) drugs or prescriptions, cosmetics (e.g. soap, shampoo, hair sprays, sunscreens, etc.) and veterinary medicines. These compounds may include hormones, antibiotics, blood lipid regulators, beta-blockers, anti-epileptics, anti-neoplastics, anti-depressants and anti-inflammatory drugs.

Once these compounds reach wastewater treatment systems, they may undergo degradation and sorption processes or remain unchanged through the system. During last two decades, pharmaceuticals and their metabolites have been detected in several sewage treatment plants effluents and surface water streams receiving the effluents (Daughton and Ternes 1999 & Daughton 2002). The main route for human pharmaceutical exposure is through effluent-dominated ecosystems (Brooks et al. 2006), while veterinary drugs are introduced to the environment via manure land application and aquaculture utilization (Boxall et al. 2004). PPCPs are consumed and discharged into sewer systems every day. Wastewater treatment plants effluents are released into surface water sources, or land applied for irrigation, fertilization or additional treatment (Davis and Cornwell 1998; Overcash et al. 2005). The irrigation water evaporates or finds its way to groundwater. Either Surface or ground water, are utilized as drinking water supplies and treated in water treatment plants. Since wastewater treatment plants have not been designed for pharmaceuticals removal, these compounds are not efficiently removed (Allaire et al. 2006; Chu and Metcalfe 2007) and there is a chance that these compounds are available in drinking water resources. Technology developments in trace elements analysis and detection methods provided the opportunity to identify these chemicals in drinking water at part per trillion (ppt or ng/l) levels (Bhandari et al. 2009). Since then, PPCPs have been evident in a wide range of environmental media e.g. fish tissue, drinking water, wastewater treatment plant effluent and sludge, soil, surface and groundwater (Kolpin et al. 2002; Alder et al. 2006; Loraine and Pettigrove 2006; Haggard et al. 2006; Karthikeyan and Meyer 2006; Vieno et al. 2007; Ternes et al. 2007; Karnjanapiboonwong et al. 2010).

Ecotoxicological Effects

Finding some ecological effects by exposure to PPCPs has caused the concern about pharmaceuticals occurrence in the environment. Reduced activity of frogs by exposure to triclosan (anti-bacterial) (Fraker and Smith 2004) and altered sex ratios in fish by exposure to estrogenic chemicals (Vajda et al. 2008) are examples of PPCPs ecological effects. Hormones are considered as Endocrine Disrupting compounds (EDCs) and are important because of their high strength even at low concentrations (Bhandari et al. 2009). Antibiotics have the potential to promote antibiotic-resistance for human pathogens. Also, they have the ability to impact microbial populations of the exposed environment, since antibiotic resistance is encoded in DNA of the microbes (Kummerer 2001). Some PPCPs such as cytostatics are important because of their destructive impacts like, carcinogenicity, toxicity and mutagenicity while other categories like analgesics are important due to their high usage amount. Behavior of these chemicals, however, in the environment at a very low concentration is largely unknown. Future research

should be conducted to identify the health effects of long-term, low-level exposures to PPCPs. As long as ecotoxicological effects of PPCPs are still not clear, assigning regulations for industries are not cost-effective and practical.

Onsite Sewage Facilities

On-Site Sewage Facilities (OSSF) are treatment systems installed at the site of one or a group of households. These systems are developed in communities where a central wastewater treatment plant is not available or is not cost effective. Approximately, one-fourth of the wastewater treatment systems are the decentralized onsite systems producing about 15 billion L/day of wastewater (USEPA 2005; Conn et al. 2006). Wastewater is collected and discharged to the treatment system typically including a septic or an aeration basin. The treated effluent is distributed over land via either surface application or an underground infiltration system. Therefore, wastewater effluent passes through several removal processes within the soil such as filtration, sorption and degradation. Several studies have investigated the presence of PPCPs in groundwater and surface waters receiving septic tank effluents (Dougherty 2011; Carrara et al. 2008; Godfrey et al. 2007; Hinkle et al. 2005; Standly et al. 2008; Wilcox et al. 2009; Wu et al. 2009) and a few researches have focused on parameters affecting the removal rates (Conn et al. 2006; Swartz et al. 2006; Stanford et al. 2010).

Few researchers have focused on PPCPs occurrence in soils and groundwater at the site of wastewater effluent land application. Ternes et al. (2007) promoted a study on a land where has been irrigated using wastewater effluent for more than 45 years. The respective soil consisted of low amounts of clay and organic carbon, so adsorption would not be the major removal procedure and biodegradation within soil explains high removals of PPCPs in groundwater. However, they detected four pharmaceuticals of 52 selected drugs in groundwater samples. In another study, Lubbock Land Application Site (LLAS) was discovered to have PPCPs in soil and groundwater by Karnjanapiboonwong et al. (2010) and PPCPs were detected in groundwater samples within the range of ND-1745 ng/l. Dougherty et al. (2010) detected 12 out of 25 compounds of interest in surface and ground waters of a coastal community using septic systems. Drewes et al. (2002) studied two full-scale water reuse facilities with surface spreading basins for groundwater recharge. They found that some antiepileptic drugs such as carbamazepine and primidone did not show significant removals after eight years. Other studies have been focused on raw wastewater application site or bio-solids land application areas, which contain higher concentrations of PPCPs, applied to the land (Gibson et al. 2010; Xia et al. 2005).

Fate and Transport in Soil

After discharge to the wastewater treatment plant, biodegradation is the dominant process for pharmaceuticals removal, while chemical oxidation and adsorption are more important in water treatment systems. Fate of PPCP within conventional wastewater treatment systems have been studied widely (Ternes 1998; Karthikeyan and Meyer 2006; Vieno et al. 2007; Ternes et al. 2007; Andreozzi et al. 2003). Depending on treatment process, compound removal may occur

through aerobic and anaerobic biodegradation, adsorption (to soil, biosolids or activated carbon), photodegradation, hydrolysis and chemical oxidation.

When wastewater is land applied it may go through photodegradation, sorption to the soil particles, biodegradation or plant uptake. Photodegradation of various pharmaceutical categories have been investigated in several studies. A vast number of compounds have been shown to be photodegraded in an aquatic microcosm study (Andereozzi et al. 2004; Latch et al. 2003). Photodegradation happens under direct or indirect mechanisms. Direct photodegradation takes place when the compound molecule absorbs solar light, whereas natural photosensitizers are causing indirect photodegradation. These chemicals such as nitrate and humic acids produce hydroxyl radicals or other strong oxidant species under solar irradiation (Zepp et al. 1981). Light intensity, temperature (Alexy et al. 2004), presence of other compounds (Doll and Frimmel 2003), dissolved organic matter (DOM) (Andereozzi et al. 2003; 2004; Doll and Frimmel 2003), concentration of nitrate ions (Andereozzi et al. 2003; 2004) and pH (Arnold et al. 2003; Andereozzi et al. 2004) are the factors that may stimulate or decrease photodegradation rates. Photodegradation of some compounds such as carbamazepine and clofibric acid may result in formation of degradation products (Doll and Frimmel 2003).

Sorption of pharmaceuticals to the soil particles is highly dependent on chemical characteristics of each compound as well as soil properties. Sorption may happen through different mechanisms including ion exchange, surface adsorption to clay minerals, hydrogen bonding and development of compounds with ions such as $Ca^{2+,}$ Mg^{2+}, Fe^{3+}or Al^{3+} and attachment to organic matter (OM) (Diaz-Cruz et al. 2003). Therefore, behavior of the compound in the environment could be estimated from parameters such as n-octanol-water partition coefficient (Log K_{ow}), acid dissociation constant (pKa) or Solubility.

Soil components such as organic matter content, pH, clay content and presence of ions may affect the sorption process (Monteiro and Boxall 2010). The pKa of each compound equals the pH in which the compound dissociation is at the state of equilibrium. Depending on pKa value and pH of the soil, the compound will be protonated or deprotonated. If pKa value of the compound is below the soil pH (acidic compounds), it will be deprotonated to reach the equilibrium conditions, so it is mostly present in anionic form in the soil. For basic compounds (pKa > soil pH), it works in reverse and they will be protonated and available in cationic forms in soil. Since OM and clay particles are negatively charged, cations will strongly adsorb to the soil and anions will be repelled. Clay content of the soil has a high sorption capacity due to its small pore sizes and large specific area (McGechan and Lewis 2002).

Soil biodegradation of pharmaceuticals is affected by soil type, temperature and moisture content (Topp et al. 2008; Collucci et al. 2001). Since PPCPs are present in the environment at very low concentrations and sorption to soil is one of the major removal procedures, they are not usually bio-available to microbes (Onesios et al. 2009). Therefore, pharmaceuticals are not consumed as the major carbon and energy source for microbial populations in the soil and their degradation is considered to be through co-metabolism (Ternes and Joss 2006), whereas enzymes produced by other metabolism processes are capable of degrading pharmaceutical compounds.

Wastewater when applied on a vegetated land, plant uptake of the pharmaceuticals is one of the removal procedures. Liquid components of the soil matrix moves into the plant so, solubility of the compound is an important factor for plant uptake. Organics with moderate hydrophobic characteristics ($0.5 < Logk_{ow} < 3$) are more likely to be translocated into plant upper-root tissues (Wenzel et al. 1999; Dietz and Schooner 2001) in the other hand, very hydrophobic compounds (log Kow >3.5) are bound to the soil organic matter or root surface and are not efficiently translocated to the other parts of the plant. In recent studies, pharmaceuticals were investigated to translocate to the upper-root sections (Boxall et al. 2006; Wu et al. 2010; Card et al. 2012). The process of plant uptake is also dependent upon the soil organic carbon content and plant lipid content. The higher the lipid content of the plant, the higher plant uptake has been observed in a study by Gao et al.(2006) and the lower the organic carbon content of the soil the higher plant uptake have been seen by Macherius et al. (2012 a). Card et al. (2012) reported that the concentrations of the hormones in the shoots are less than model predicted amounts suggesting availability of some transformation procedures. Once compounds are taken up, they may move into plant tissues via transpiration and then volatilize or completely (or partially) degrade (Briggs et al. 1982; Chiou et al. 2001; Li et al. 2005; Su and Zhu 2007; Alkorta and Garbisu 2001; Karnjanapiboonwong 2011). Phase II metabolism of the antibiotic agent triclosan was seen to occur in carrot (Macherius et al. 2012 b).

Acetaminophen

Acetaminophen or Paracetamol is an over the counter (OTC) anti-inflammatory drug commonly used as a pain and fever reliever and it can be found in many cold and flu prescriptions as the main ingredient. Acetaminophen has been detected in wastewater treatment plant effluent (Boyd et al. 2003), surface and ground waters (Kolpin et al. 2002), soil (Kinney et al. 2006), agricultural field runoff (Pedersen et al. 2005) and drinking water (Boyd et al. 2003).

When released to the environment, sorption of this compound to the soil and sediments is not expected to be an important removal procedure based on its low log K_{ow} value. Acetaminophen is considered highly mobile in soil environment, since it is moderately soluble in water. Acetaminophen pKa value of 9.38 indicates that this compound will partly exist in cation form (as explained above) in soil pH and since clay and organic carbon existing in soil have the negative charge, they attract each other and this compound is expected to adsorb moderately to soil containing high clay content. Half-life of 16-26 hrs indicates that acetaminophen is easily biodegradable in the environment (Lam et al. 2004). In a lab study performed by Lam et al. in 2003 showed that photo degradation plays an important role in acetaminophen removal (Lam et al. 2003).

Carbamazepine

Carbamazepine is an anti-convulsant or anti-epileptic drug, which is prescribed alone or combined with other medicines. It is used as a mood-stabilizing drug to control certain types of seizures or treat trigeminal neuralgia. This compound is the most detected drug in the environment in anti-epileptics category and have been identified in several environments such as

sewage treatment plant effluent (6.3 ug/L by Ternes 1998), sludge (20.9 ng/mg, Fent et al. 2006), surface waters (1 ug/l by Wiegel et al. 2004, Ternes et al. 1998 and Heberer 2002), sediments (4.2 ng/mg by Thaker 2005), soil and biosolids (Kinney et al. 2006 a&b), runoff (0.44 ug/l by Pedersen et al. 2005), groundwater (Seiler et al. 1999, Sacher et al. 2001 and Ternes et al. 2001; 2007) and drinking water (0.018 ng/l by Benotti et al. 2009).

Carbamazepine when released to the environment has a moderate tendency to adsorb to the soil and sediments with regard to its log Kow value of 2.45. Therefore, this compound will be moderately mobile in the soil. However, it has also been classified as slow-mobile compound in organic matter-rich soil types (Chefetz et al. 2008). Carbamazepine is not volatile from moist soil surfaces given an estimated Henry's Law constant of 1.1×10-10 atm-cu m/mole. Due to the lack of functional groups, carbamazepine is not hydrolyzed easily under environmental conditions (Lyman et al. 1990). Studies on photodegradation of carbamazepine represent its high half-life in outdoor microcosms (82 d, Lam et al. 2004) and natural river water (38 d, Andreozzi et al. 2002) systems. Slow biodegradation rate (A 7% removal rate in wastewater treatment plants Doll et al. 2003) and a biological half-life of 63 days in lake water (Tixier et al. 2003), indicates that this compound is hard to degrade in the environment and may be persistent in several circumstances to reach drinking water. Oppel et al. (2004) did not find any difference in sorption behavior of carbamazepine by changing soil pH and organic carbon content and it was not detected in the leachate of 30cm soil columns irrigated with pharmaceuticals contaminated water. Carbamazepine however, was detected in the groundwater tests (Drewes et al. 2002; Sacher et al. 2001; Heberer 2002). In a soil column study, carbamazepine was detected in the leachate at the concentration of 0.116 µg/l. Columns were loaded 20 days prior to sampling with 0.170 µg/L of carbamazepine mixture (Cordy et al. 2004). Stackelberg et al. (2004) discovered carbamazepine persistence through water treatment units such as coagulation, flocculation, activated carbon adsorption, filtration, and disinfection, and it was detected in finished drinking water at low µg/l levels.

Fluoxetine

Fluoxetine is an anti-depressant used to treat major depressive or obsessive-compulsive disorders. It is also one of the selective serotonin reuptake inhibitors (SSRIs). Fluoxetine appears to have the lowest value of acute toxicity among human drugs reported so far and based on comparison of environmental concentrations and chronic toxicity of pharmaceuticals, lowest observed effect concentration (LOEC) of fluoxetine for zooplankton and benthic organisms were close to highest wastewater treatment plant effluent concentrations detected (Fent et al. 2006). In the other study it was shown that development of tadpoles was delayed even at fluoxetine concentrations of 0.012–0.099µg/L which is common in the environment (Foster et al. 2010). It can be present in municipal wastewater as a contaminant and has been detected in wastewater treatment plant effluent (99 ng/l by Metcalfe et al. 2003), soil (Kinney et al. 2006), US streams (12 ng/l by Kolpin et al. 2002), drinking water (Benotti et al. 2009) and fish tissue (Brooks et al. 2005).

If Fluoxetine is available in the environment, based on its high log K_{ow} value of 4.65, adsorption to the soil and sediments in water bodies will be the main fate process of this compound. Therefore, Fluoxetine appear to have low mobility in the soil environment. This organic base has a pKa =10.05 (Vasskog et al. 2006), representing that it will be present in cation forms at environmentally relevant pH values (Kwon and Armbrust 2006). Kwon and Armbrust (2008) discovered that fluoxetine shows negligible photodegradation, hydrolysis or microbial degradation in aquatic systems and adsorption to the sediments is the main removal procedure. High solubility, high log K_{ow} resulting in high organic carbon partition and high pKa value resulting in ionic binding explains its high affinity to sorption.

Trimethoprim

This compound is one of the antibiotics used in both human and veterinary medicines (Boxall et al. 2006). It is normally used as a prescription to ear or bladder and urinary tract infections. Trimethoprim has been detected in wastewater treatment plant effluent (0.66 ug/l, Hirsch et al. 1999), drinking water (Snyder et al. 2007), soil (Kinney et al. 2006), agricultural field runoff (Pedersen et al. 2005) surface and groundwater (Ashton et al. 2004; Hilton and Thomas 2003; Hirsch et al. 1999).

If trimethoprim is released to the environment, it will remain in the air as a particulate matter based on its low vapor pressure. Volatilization of this compound is not expected in any environment. This compound will have a high mobility in soil regarding its log K_{ow} value of 0.91. pKa of trimethoprim is 7.12, which is near soil pH and indicates it will somewhat be present in protonated form in moist soils, but mostly in neutral form since the pKa value is near soil pH. Environmental half-life of trimethoprim is more than 30 days (Boxall et al. 2004), therefore the biological degradation of this compound is slow. While photolysis half-life for trimethoprim is about 6 days, biodegradation does not appear to be an important factor for fate of this compound in the environments exposed to sunlight (Lam et al. 2004).

Significance of the Study

Within the last decade, several researchers have conducted studies to investigate the occurrence, fate and removal of PPCPs. Many of the experiments however, were established in laboratories with predetermined variables and conditions, so pharmaceutical behavior assessment in soil lacks pilot-scale studies where all removal procedures take place. Therefore, there are needs for research prior to providing any regulations or guidelines for wastewater treatment plants management regarding PPCP issues. In this research, fate and removal of the pharmaceuticals in a pilot-scale OSSF is examined. It is hypothesized that the wastewater containing PPCPs can be substantially cleaned of its PPCP load by surface application and percolation through the soil profile. In addition, the potential for accumulation in soil and plants will be evaluated for each class of pharmaceuticals considered in this project. The main objectives of this research are:

I. To investigate the PPCP removal capacity of Lubbock land treatment site soil within each season of the year by using soil lysimeters

II. To determine the effect of photodegradation on pharmaceuticals removal rate

III. To determine effect of plant existence on PPCP removal rate

IV. To study the removal pathways of PPCPs by analyzing soil and plant tissue samples

V. To investigate pharmaceuticals accumulation profile inside soil columns

To accomplish the objectives of the study two project phases have been defined which are described in the following chapters. The final conclusion in provided at the end of this report. All references and original datasets are included at the end of the report.

Transport of Pharmaceuticals in Soil Following Onsite Wastewater Effluent Application

As mentioned in the introduction section, onsite wastewater treatment systems (OWTS) are de-centralized systems installed to serve one or a few number of households. Unlike centralized wastewater treatment plants, OWTS have not received much research on removal processes of pharmaceuticals. One-fourth of American households, however, use these systems (US EPA 2002). Few studies have been conducted to examine the occurrence of pharmaceuticals in groundwater associated with septic tank effluents. Carrara et al. (2008) detected 10 out of 12 target compounds at low ng/l to low µg/l concentrations, while Godfrey et al. (2006) detected 3 out of 12 pharmaceuticals in a subsurface sand and gravel aquifer recharged by septic tank effluent. Occurrence of 25 pharmaceuticals in groundwater and surface waters affected by septic tank effluent leaching was studied by Dougherty et al. (2010). Hinkle et al. (2005) detected a subset of pharmaceuticals in the groundwater at concentrations between 0.1-0.18 µg/l and indicated that some anticonvulsant drugs may serve as indicators for aquatic contamination by wastewater. Some research has focused on the effect of parameters on removal rates such as loading rate and effluent quality (Conn et al. 2010), depth and oxygen availability in soil (Swartz et al. 2006), soil type (Gielen et al. 2009), and total organic carbon and presence of surfactants on estrogen mobility in soil (Stanford et al. 2010). In addition to these parameters, method of effluent application to land remains another factor that may affect pharmaceutical transport and removal in soil.

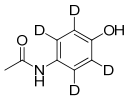
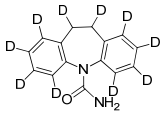
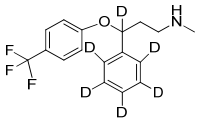
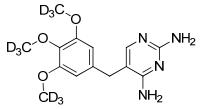
In the present study, the capacity of surface applied systems for pharmaceutical removal was determined from three different land application systems: a vegetated lawn, an Unvegetated land covered from sun and bare soil. Differences in removal were evaluated among three systems monthly over a one-year time period. The goals of this study were to (1) determine the effect of vegetation on pharmaceutical removal capacity of the wastewater land application systems (WWLAS), (2) determine the effect of photodegradation on pharmaceuticals removal in WWLAS and (3) explore how the removal of each compound varies by over the course of a year. Four model pharmaceuticals (acetaminophen, trimethoprim, carbamazepine, fluoxetine) were selected based on their physicochemical properties, biological activities and previous reports of environmental occurrence (Monteiro and Boxall, 2010; Brausch et al., 2012). To accomplish the goals of this study, a pilot-scale experiment was conducted and synthetic wastewater was used as the irrigation water.

Materials and Methods

Study Compounds

Acetaminophen, carbamazepine and fluoxetine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trimethoprim was provided from Fluka Biochemika (Switzerland). Select physicochemical properties of each compound are listed in Table 1. Stock solution of each pharmaceutical was prepared in deionized water individually at concentrations lower than water solubility of each chemical. The solutions were stored in acetone-washed amber bottles at 4° C in the dark prior to application.

Table 1. Physiochemical properties and structures of pharmaceuticals selected for study.

	Acetaminophen	**Carbamazepine**	**Fluoxetine**	**Trimethoprim**
Chemical Abstract Service (CAS) #	103-90-2	298-46-4	54910-89-3	738-70-5
Medical class	Anti-inflammatory	anti-epileptics	antidepressant	antibiotic
Molecular structure (labeled)				
Molecular formula	C_8-H_9-N-O_2	C_{15}-H_{12}-N_2-O	C_{17}-H_{18}-F_3-N-O	C_{14}-H_{18}-N_4-O_3
Molecular weight	151.16	236.27	309.33	290.32
pKa	9.86	13.94	10.06	7.12
Log K_{ow}	0.46	2.45	3.93	0.91
Solubility in water (25°C; mg/L)	14,000	18	14,000	400
Predicted Koc (pH=7)	43.1	256	5.49	23.7

(Scifinder, 2012).

Experimental Columns

Soil columns were developed by direct removal of soil from the ground at the site of the Texas Tech University's Department of Plant and Soil Sciences Farm. The soil profile and heterogeneity were kept unchanged for these test systems so that the data would represent the native soil behavior. Therefore, the soil columns were filled with water from the bottom of the test chamber twice to allow the soil particles to settle similar to field conditions. Then the columns were irrigated daily for conditioning and check for possible system errors over the next month. Soil properties are presented in Table 2. The test columns used were soil columns with 30 cm inner diameter and 90 cm in height containing 75 cm of soil and 15 cm of supporting materials (sand and gravel) at the bottom which are shown in Figure 1. The soil supporting material allow water flowing below the plant root zone to be stored within that area of the columns and collected monthly using the available discharge valve at the bottom of the columns.

Table 2. Properties of soil used in experimental design.

Property	**Organic matter (%)**	**Clay (%)**	**Silt (%)**	**Sand (%)**	**Soil Type**	**Soil pH**	**CEC* (meq/100gr)**
Value	2.3 - 2.9	24 - 32	15 - 21	50 - 58	Sandy Clay loam	8.0-8.6	15 - 17

* Cation Exchange Capacity

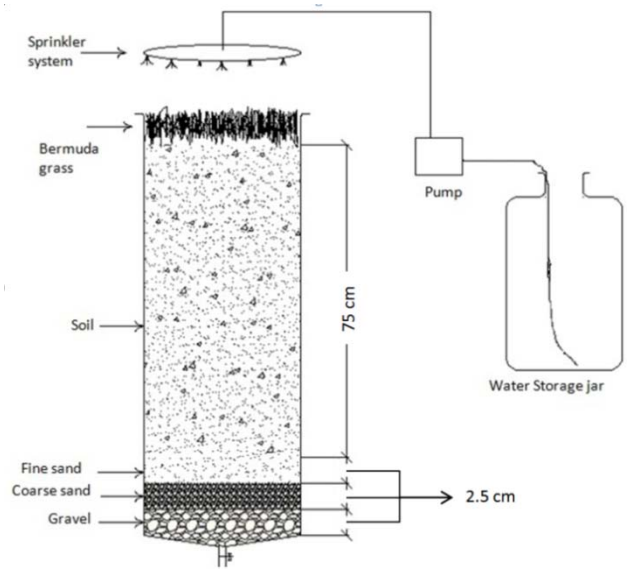


Figure 1. Schematic cross-sectional view of the grass-covered column setup.

To minimize the variables in the system, synthetic wastewater was developed, consisting of tap water, acetaminophen, carbamazepine, fluoxetine and trimethoprim, each at 100 µg/L of concentration that is compatible with previous study results on septic tank effluent analyses (Wilcox et al. 2009) along with Miracle Grow to provide 25 mg/L of total nitrogen.

Experimental Design

Three different treatment levels of soil lysimeters, each with three replicates, were used to determine the effect of grass and UV radiation on removal rates of the four pharmaceutical compounds chosen for study. Group one consisted of columns with Bermuda grass, Cynodon dactylon on top (Figure 1), whereas the second group consisted of plastic-covered columns to avoid any evaporation and solar radiation. The third group consisted of the columns with bare soil on top that was exposed to natural sunlight. Hereafter, these experimental factors are referred to as the grassed columns, the covered columns and the open columns. Bermuda grass was used because it is one of the most common lawn grasses used in west Texas. The height of the grass was maintained at five centimeters to avoid evapotranspiration variability within each season. Starting in January 2010, synthetic wastewater was applied over the soil columns using a spray irrigation system on top of each test column. A peristaltic pump was used to transport water from the supply tank to the irrigation system. The pumping schedule was adjusted using a timer in a way that columns were irrigated for 10-20 min at 5:00 AM to meet the Texas environmental regulation for irrigation of onsite effluent (Texas Administrative Code 2006). Loading rates were adjusted for each month to meet the evapotranspiration (ET) rates and have at least 1 liter of effluent to be collected for laboratory analysis. The system was operated similar to the actual land application site where leaching may or may not occur during the different seasons. This study continued for one year.

Sample Collection

All columns were drained overnight every month; 1 L- volume samples were collected in amber bottles, packed and shipped on ice to Baylor University for analysis of leachates and synthetic wastewater analysis. The following steps were used to determine the concentration of target pharmaceuticals present in leachate and influent samples (Du et al. in review; Watson et al. in review).

Sample Extraction

Prior to extraction, samples were prepared by adding ascorbic acid (at 50 mg/l concentration) to samples of 500 ml volume to satisfy any residual oxidants such as ozone, chloramines and chlorine. Filter papers, 0.45 and 0.2 μm, were used sequentially to remove excess solids, minimizing the impact on solid phase extraction (SPE) efficiency. Same aliquot of isotope-labeled analogs mixture (100 ng/l) was added to both calibration sample and collected water samples. Analytes were extracted using 5 mL, 200 mg hydrophilic-lipophilic balance (HLB) glass cartridges from Waters Corporation. All extractions were performed on an Auto Trace automated SPE system. The SPE cartridges were sequentially preconditioned with 5 mL of MTBE, 5 mL of methanol, and 5 mL of nano pure water. The samples were then loaded onto the cartridges at 2 mL/min. Next, the cartridges were air-dried and eluted with 5 mL of methanol followed by 5 mL of 10/90 (v/v) MeOH/MTBE into culture test tubes. The resulting extract was concentrated with a gentle stream of nitrogen to dryness. The extract was brought to a final volume of 1 mL using 5:95 MeOH: HCOOH (0.1%). Prior to analysis, samples were sonicated for 1 min and filtered using Pall Acrodisc hydrophobic Teflon Supor membrane syringe filters (13-mm diameter; 0.2-μm pore size).

LC-MS/MS Analysis

Analytes were separated on a 15 cm × 2.1 mm (5μ m, 80 Å) Extend-C18 column (Agilent Technologies, Palo Alto, CA) connected with an Extend-C18 guard cartridge 12.5 mm x 2.1 mm (5 μm, 80 Å) (Agilent Technologies, Palo Alto, CA), employing a Varian ProStar Model 212 pump system equipped with a Model 410 autosampler. A binary gradient consisting of 0.1% (v/v) formic acid in water and 100% methanol was employed to achieve chromatographic separation. Additional chromatographic parameters were as follows: injection volume, 10 μL; column temperature, 30 ºC; flow rate, 350 μL/min. Eluted analytes were monitored by MS/MS using a Varian model 1200L triple-quadrupole mass analyzer equipped with an electrospray interface (ESI).

Calibration and Method Detection Limits (MDL)

An isotopic labeled version of each analyte, corresponding to the isotopes added to each sample prior to extraction, was added to each calibration point at a concentration of 100 μg/L to generate a relative response ratio. Recoveries of the isotopes were compared with the relative response ratio and a concentration for the unlabeled analyte was calculated. Linear or quadratic regression $r^2 \geq 0.998$ was used for all analytes. Instrument calibration was monitored through the use of continuing calibration verification (CCV) samples with an acceptability criterion of ±20%.

In a given run, one CCV sample was interspersed between every twelve samples for quality assurance purposes. In present study, MDLs represent the lowest concentration of analyte that may be reported in each respective sample matrix with 99% confidence that the concentration is different from zero. MDLs for acetaminophen, trimethoprim, carbamazepine and fluoxetine were 1.3, 0.95, 0.51 and 7.9 ng/L, respectively.

Mass Balance Analysis

In order to determine the removal efficiencies and mass of study pharmaceutical removed in each column, a mass balance calculation was determined for all compounds according to Equations (1) & (2).

$$MR = C_{in} \times V_{in} - C_{out} \times V_{out} \quad (1)$$

$$MRR(\%) = [C_{in} \times V_{in} - C_{out} \times V_{out}] \times 100 / [C_{in} \times V_{in}] \quad (2)$$

Where, MR = Mass of chemical removed,

MRR(%) = Mass removal rate,

C_{in} = Pharmaceutical concentration in the influent,

C_{out}= Pharmaceutical concentration in the leachate,

V_{in} = Monthly loading water volume,

and V_{out} = Monthly leachate water volume.

Statistical Analysis

Experimental results were statistically analyzed using SAS software (SAS, 9.3) and level of significance was $p < 0.05$. The experimental design was a factorial completely randomized design (CRD), where the type of the column and the month of the year were the main factors. Since the main effects, effect of month or column type, were not significant in the proc GLM procedure, multiple comparisons procedures using mean separation tests, for each pharmaceutical, on both column treatment levels and months were performed using Tukey's method. Non detected points were replaced with the half of the MDL values. In one other test each three months were grouped in one season and the same analysis was run to discuss the effect of season. All the tests were performed on both leachate concentrations of each compound and the calculated removed mass of each chemical from the columns.

Results

PPCP Leachate Concentrations

Acetaminophen, trimethoprim, carbamazepine and fluoxetine were detected at the ranges of ND-540 ng/L, ND-1200 ng/L, ND-750 ng/L, and ND-968 ng/L, respectively. Figures 2 to 5 show the mean detected concentrations of study compound in each group of column type over the

course of the experiment. The missing data points are associated with the high temperature seasons where volumes of the leachate from the columns were insufficient for laboratory analysis. The relatively higher concentration of acetaminophen, trimethoprim and carbamazepine were detected in the grassed and open treatment level leachates. Although fluoxetine was not detected in any of the leachate samples from the grassed and open treatments, it was detected at relatively high levels in covered columns in July and December. Trimethoprim and carbamazepine were the most frequently leached compounds throughout the year and were detected at higher concentrations in leachates from grassed and open columns, whereas acetaminophen and fluoxetine were not detected in some samples (Figures 2 to 5). Calculations on mass removal rates result in more than 95% removal rates in all columns; however, the results indicate that groundwater receives a certain amount of pharmaceutical discharged daily (Table 3). Based on the experimental approach employed in the present study with soil columns, the presence of grass generally appears more effective for reducing introduction of these target analytes to groundwater during spring and summer seasons, whereas converse observations may be expected during winter and fall months for this soil type.

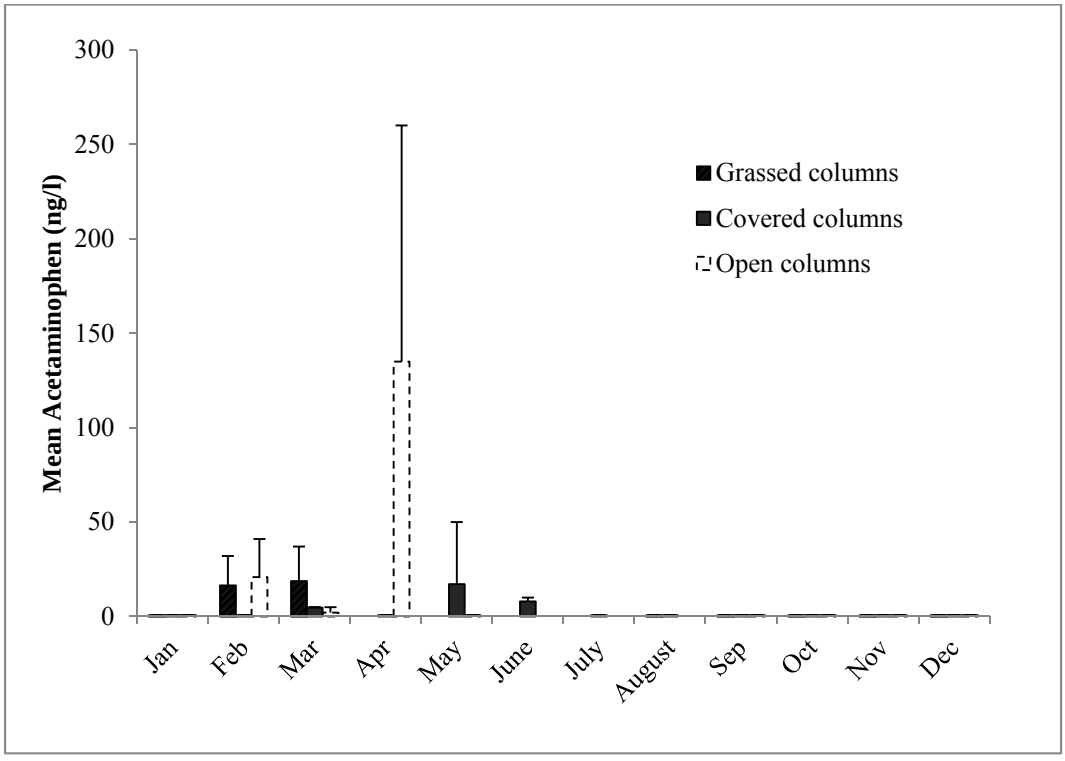


Figure 2. Mean + standard deviation (n = 3) acetaminophen concentrations in soil column leachate from grassed, covered and open columns. Half of the minimum detection limit (0.66 ng/l) was used for the non-detected values.

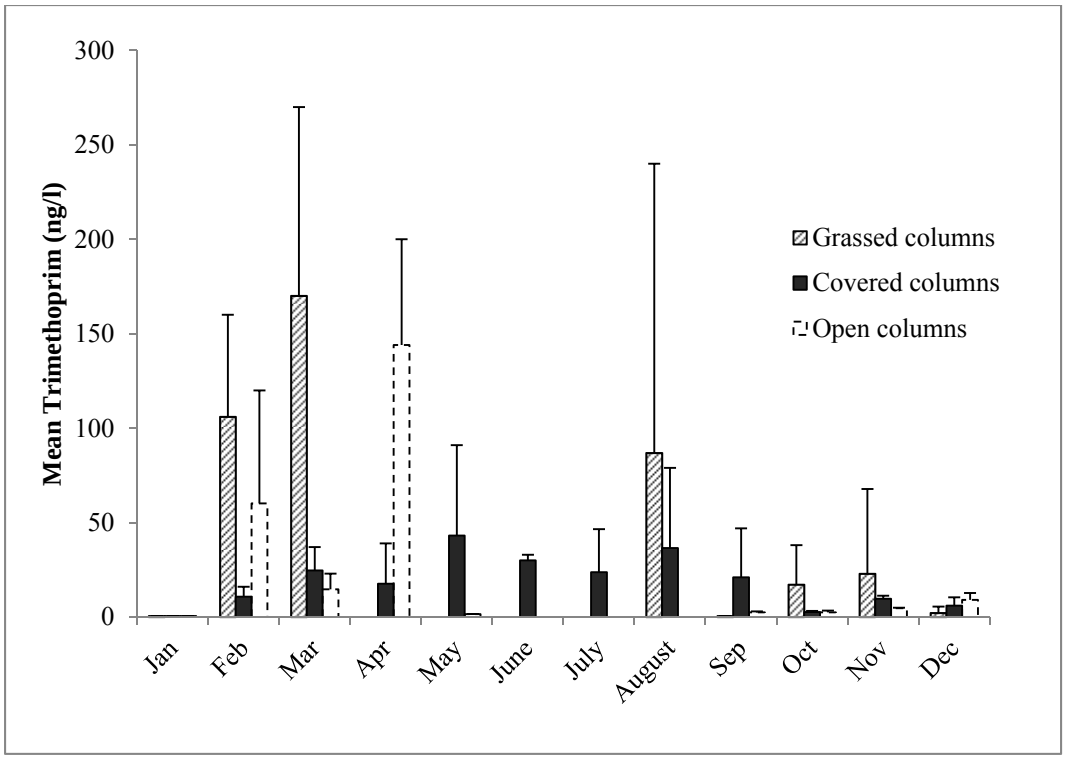


Figure 3. Mean + standard deviation (n = 3) trimethoprim concentrations in soil column leachate from grassed, covered and open columns. Half of the minimum detection limit (0.47 ng/l) was used for the non-detected values.

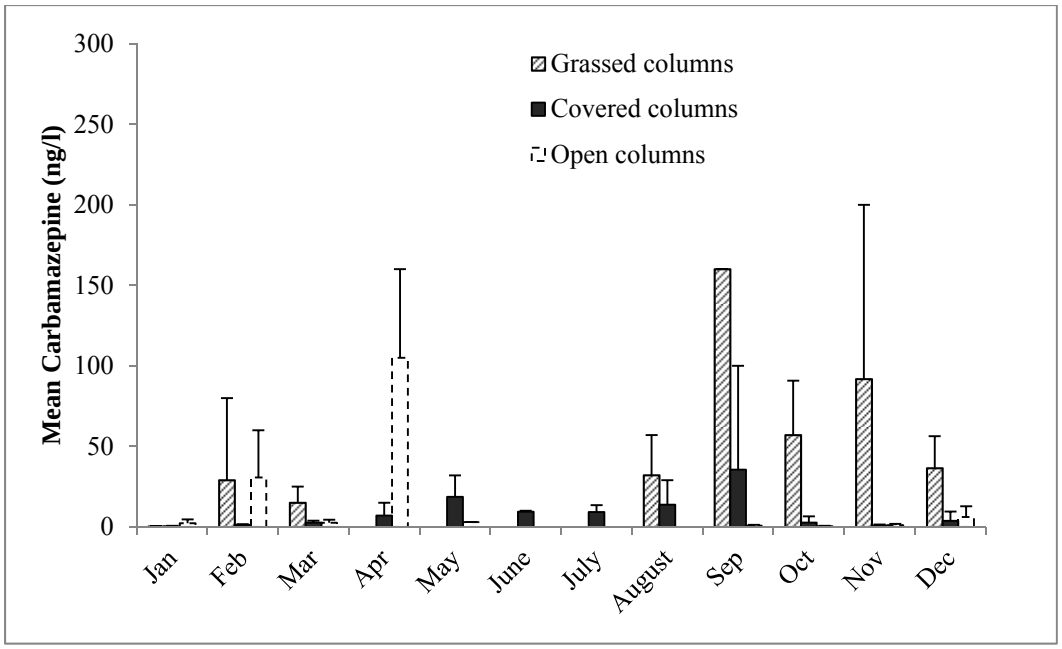


Figure 4. Mean + standard deviation (n = 3) carbamazepine concentrations in soil column leachate from grassed, covered and open columns. Half of the minimum detection limit (0.25ng/l) was used for the non-detected values.

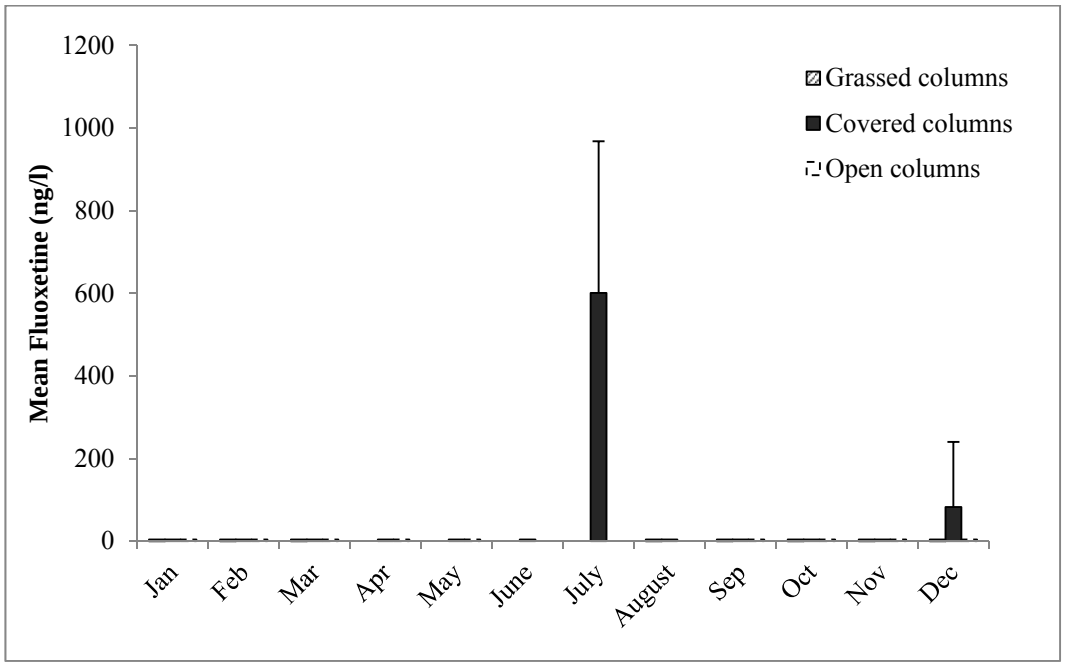


Figure 5. Mean + standard deviation (n = 3) fluoxetine concentrations in soil column leachate from grassed, covered and open columns. Half of the minimum detection limit (3.97 ng/l) was used for the non-detected values.

Table 3. Calculated mean daily discharge by season of target pharmaceuticals from soil columns per liter of applied wastewater (ng/m^2/L/day).

	Spring			Summer			Fall		
Pharmaceutical	**Grassed**	**Covered**	**Open**	**Grassed**	**Covered**	**Open**	**Grassed**	**Covered**	**Open**
Acetaminophen	2.2	10	0.5	3.4	4.1	0.01	0	0.03	0.04	0.02
Trimethoprim	12	22	2.5	5.9	4.3	6.6	0	5.8	2.5	0.3
Carbamazepine	7.6	14	0.8	2.2	3.7	1.5	0	15	6	0.1
Fluoxetine	0.5	4	0.2	0.2	0.2	0.03	0	0.2	0.2	0.2

Winter

Pharmaceutical Removal

Mass balances were determined for study compounds in each soil column to calculate the mass removed each month throughout the year. Whenever zero leaching was observed, the amount of pharmaceuticals applied to each column throughout the month was calculated and considered to be the removed amount. The mass removed in different column types were compared to each other for each month using multiple comparison method. The effect of column type treatment was significant ($p<0.05$) in some months and was not in some other months. Because of the large amount of data, only the number of months in a season that the effect of column type in removed amount was significant is presented in Table 4. Comparison of the grassed columns and covered columns shows higher number of significant months. The results indicate that the grassed columns were more frequently different from covered and open columns suggesting that mass of pharmaceuticals removed was greatest within the grassed columns, followed by the open, and lastly the covered columns.

The effect of loading rate (Table 5) by season needs to be considered in the analysis because it changes due to crop ET and evaporation. During warmer seasons grassed and open columns required more water compared to covered columns and still leaching was minimal. During winter and spring all column types received nearly the same amount of water, so the results shown in Table 4 represent the effect of column cover for these two seasons. Since the spring leachate was zero from grassed columns, the calculated mass removed became the same as inlet mass which explains the insignificant difference between column types in spring season. The inlet water volume applied was different for different column types in summer and fall resulting in significant differences in compound removal (Table 4).

Table 4. Number of month in each season that represents significant ($P < 0.05$) column type effect in pharmaceuticals mass removed.

	Grassed vs. Covered				Covered vs. Open				Grass vs. Open			
Compound	**Winter**	**Spring**	**Summer**	**Fall**	**Winter**	**Spring**	**Summer**	**Fall**	**Winter**	**Spring**	**Summer**	**Fall**
Acetaminophen	3	0	3	3	1	0	1	3	2	0	3	2
Carbamazepine	3	0	3	3	1	0	2	3	3	0	3	2
Trimethoprim	3	2	3	3	1	1	2	3	3	1	3	1
Fluoxetine	3	1	3	3	1	0	2	3	3	1	3	2

Table 5. Average water loading rates on each column type (L/month).

Column type	**Winter**	**Spring**	**Summer**	**Fall**
	Loading rates (L/month)			
Grass	11.5	6.4	12.3	12.0
Covered	9.5	6.2	5.5	4.7
Open	10.4	6.2	8.2	11.4

After analysis of the removed mass on a month by month basis, the overall mass removed by the end of the year was also calculated. Although monthly leaching concentration data did not show any significant trend for the removal of the tested compounds, the overall removed mass represented a considerable trend within column types. The total mass removed throughout the year in the grass columns were significantly higher than other columns (P-value <0.05) and the mass removed by the open columns were significantly higher than covered columns (P-value<0.05), for all compounds tested (Table 6). This comparison shows more than 30% and 15 % increase in removed mass by the grassed columns rather than covered columns and open columns, respectively. These numbers are slightly different for each compound.

Table 6. Mean removed mass of the various compounds in each column type (g). (n=3).

Column Type			
Removed Mass (g)			
Compound	**Grassed**	**Covered**	**Open**
Acetaminophen	1.8± 0.0*	1.3 ± 0.0	1.7 ± 0.0
Trimethoprim	17.6 ± 0.05	10.6 ± 0.04	14.4 ± 0.02
Carbamazepine	7.5 ± 0.02	4.2 ± 0.04	6.1 ± 0.01
Fluoxetine	7.1 ± 0.02	4.7 ± 0.02	6.1 ± 0.04

*Mean± Standard deviation.

Discussion

Several factors can influence fate and transport of pharmaceuticals applied to soils from onsite wastewater. Sorption of pharmaceuticals to soil is highly dependent on chemical characteristics of each compound and site-specific soil properties. Therefore, behavior of the compound in the environment could be estimated from parameters such as log K_{ow}, acid dissociation constant (pKa) and/or solubility. Further, organic matter (OM), pH, cation exchange capacity (CEC), clay and various ions may affect sorption processes (Monteiro and Boxall 2010). Depending on pKa value and pH of the soil, the compound will be protonated or deprotonated. If pKa value of the compound is below the soil pH, the compound is deprotonated and present in anionic form in the soil. Because OM and clay particles are negatively charged, cations strongly adsorb to the soil and anions are repelled. In addition, clay content of the soil has a high sorption capacity due to its small pore sizes and large specific area (McGechan and Lewis 2002).

To examine such influences of physicochemical properties on soil absorption, we selected pharmaceuticals for the present study that ranged in physicochemical properties known to influence soil sorption. For example, carbamazepine, a common antiepileptic, is a neutral pharmaceutical that is moderately hydrophilic (log K_{ow} = 2.45). Acetaminophen, a nonsteroidal antiinflamatory, is a weak acid that is hydrophilic (log K_{ow} = 0.4) and ionizable at environmentally relevant pH (pKa = 9.38). Trimethoprim, an antibiotic, and fluoxetine, an antidepressant, are weak bases that differ in pKa and log P values. Whereas trimethoprim is more hydrophilic (log P = 0.91) with a circumneutral pKa (7.12), fluoxetine is more lipophilic (log Kow = 4.65) with a higher pKa (10.06). Several previous studies have examined the sorption dynamics of these compounds in various soil types. Of particular importance, Williams et al (2006, 2009) demonstrated that volume of distribution (Vd), an important pharmacokinetic

parameter for pharmaceuticals, may be used to predict partitioning of ionizable therapeutics in soil systems. Thus, carbamazepine represented an important exception (Williams et al 2006, 2009). This stands to reason because Vd describes a volume in which a pharmaceutical is distributed within the body; this higher the value, the greater the partitioning of a drug into other compartments (e.g., lipids). For example, the Vd of fluoxetine is an order of magnitude higher (e.g., 2450) than the other compounds examined in this study. Thus, our study observations generally support those of Williams and colleagues (2006, 2009), because fluoxetine was consistently removed to the greatest extent by soil columns in the present study, regardless of soil column type or season.

Photodegradation of various pharmaceutical categories have been investigated in several studies. A vast number of compounds have been shown to be photodegraded in an aquatic microcosm study (Buth et al. 2009; Packer et al. 2003; Andereozzi et al. 2004; Latch et al. 2003). Regarding Figure 2, acetaminophen concentration is lower for open columns in the month of May, which may be a result of photodegradation in the open columns, since there are more sunny days in May compared to previous months and a previous lab study showed that photo degradation plays an important role in acetaminophen removal (Lam et al. 2003). It can be seen that acetaminophen was not detected in leachate after the month of July; this may be a sign of microbial population build-up in soil, since acetaminophen is highly biodegradable (Lam et al. 2004). Acetaminophen is moderately soluble in water which will help its mobility throughout the soil matrix, however the clay content of the soil is high as well as the pKa value of the compound resulting in more bonding in soil and remaining in the system. The longer acetaminophen ions stay in soil, the more the microbes will be adapted to utilize it. While acetaminophen has been removed to a high extent, trimethoprim and carbamazepine have been detectable in the leachate (Figures 3 and 4) within all months during the project period. According to the chemicals characteristics, provided in Table 1, trimethoprim has low water solubility, low Log (K_{ow}) value, neutral pKa, and long biological half-life. Therefore, the principal removal process for trimethoprim is mainly based on filtration. Also, trimethoprim is not passing through the soil in a soluble form, has low tendency to adsorb to the soil organic matter and is biodegraded slowly. Trimethoprim showed similar results to carbamazepine in having the highest frequency of detection.

Slow biodegradation rate (A 7% removal rate in wastewater treatment plants. Doll et al. 2003) and a biological half-life of 63 days in lake water (Tixier et al. 2003), indicates that carbamazepine is hard to degrade in the environment and may be persistent in several circumstances to reach drinking water. Regarding Figure 4, carbamazepine concentration in leachate has a reverse relation with plant viability during seasons of the year. Hence, available plants at the land application site could be effective in removal of carbamazepine. Liquid components of the soil matrix move into the plant, so solubility of the compound is an important factor for plant uptake. Also organics with moderate hydrophobic characteristics ($0.5 < \text{Log}(K_{ow}) < 3$) are more likely to be taken up by plants (Wenzel et al. 1999). Carbamazepine has log (K_{ow}) value of 2.45 which is in the range of high affinity for plant uptake. In the fall season when plant uptake decreases, carbamazepine concentrations in leachate increase. This relation is not noticeable for the covered columns which lack plant presence.

Figure 5 shows that high removal rates are also obtained for fluoxetine in all columns. Fluoxetine may reach the groundwater if it is not degraded while passing through soil or not adsorbed onto soil or organic matter. This compound has a high log (K_{ow}), which indicates, it can be adsorbed to organic matter quickly. Within the month of July, fluoxetine concentrations in leachate are high, which may be a result

of accumulation and desorption after a given amount of time. This would not have happened if fluoxetine was biodegraded at a high rate within the previous 5 months.

Concentrations of the target pharmaceuticals are consistent with the ones detected in groundwater associated with septic tank effluent discharge stated in previous studies (Godfrey et al. 2007 and Dougherty 2011). The comparison of the column types based on leachate concentrations did not show any significant difference among column types, which is a result of soil matrix complexity and missing data due to varying weather conditions. Even though the soil lysimeters were established from the same soil, each column may vary to a small degree based on different degrees of channeling and biological activities, which have been discussed to have a high impact on experimental results variations (Enell et al. 2004).

PPCPs Accumulation in Soil and Plant Following Wastewater Land Application

Environmental occurrence of the Pharmaceuticals and Personal Care Products (PPCPs) has been a concern over last two decades once some ecotoxicological risks regarding presence of these compounds were determined (Fraker and Smith 2004; Vajda et al. 2008). Since then, researchers have been focusing on the fate and removal of these compounds within wastewater treatment plants (WWTPs), which are apparently not very effective in removal of some compounds (Ternes 1998; Zhang et al. 2008). Since wastewater reuse is a critical procedure when the need for water is increasing, it is land applied after primary or secondary treatment either from centralized or decentralized systems for agricultural or landscaping purposes. WWTP nutrient-rich sludge will also be land applied as a fertilizer for soil nourishment. These reuse sources can contain pharmaceuticals at levels ranging from ng/l in water to µg/g in biosolids (Ternes et al. 2007; Martin et al. 2012). Soil acts as the secondary treatment system to remove water contaminants such as salts, nitrogen and phosphorous (Duan and Fedler 2011). Dissipation of the pharmaceuticals in soil is a result of several procedures such as sorption and biodegradation (Boxall and Ericson 2012) affected by characteristics of the compound as well as soil properties. Some of the compounds such as carbamazepine are found to be very persistence in the environment showing removal rates less than 10% in WWTPs (Ternes 1998; Zhang et al. 2008) and low kd values for sorption to sludge (Ternes et al. 2004; Scheytt et al. 2005) or sandy soil (Gielen et al. 2009). Uptake of carbamazepine by edible vegetables and crops has been studied previously and pharmaceuticals translocation to different parts of the plants and human daily intake of each compound resulting from the crop consumption was estimated (Boxall et al. 2006, Shenker et al. 2011; Winker et al. 2010; Calderón-Preciado et al. 2011; Wu et al. 2010 and Herklotz et al. 2010).

Major removal rates of pharmaceuticals and personal care products were observed while these chemicals were passing through soil along with a wastewater stream, however only a few of the compounds have been detected in groundwater (Ternes et al. 2007; Focazio et al. 2008; Loos et al. 2010). The sorption of the pharmaceuticals by soil could be a great removal process preventing groundwater contamination but still there is a risk of bioaccumulation of these chemicals in soil organisms such as worms as reported by Kinney et al. (2008) and transported through the food chains. In previous studies, while major concern was on groundwater pollution, soil contamination has not received adequate attention in a scale larger than laboratory batch experiments.

Potential chemical uptake by plants grown at the site where wastewater is land applied should be more completely understood. In this study, Bermuda grass, Cynodon dactylon, uptake of four selected pharmaceuticals, acetaminophen, trimethoprim, carbamazepine and fluoxetine, was investigated as a removal procedure as the wastewater moves through the soil beyond the root zone of the plants and three different wastewater land application media, grass-covered lawns, any unvegetated land covered from sunlight and bare land, were compared in terms of their potential for target compounds accumulation in soil. The transport of these pharmaceuticals was evaluated to discover (1) the accumulation pattern in the soil profile and (2) uptake of the target compounds by grass

Materials and Methods

Soil and Plant Sample Collection

Since the removal rates of all of the target compounds in the leachate and for all columns tested were higher than 95% (chapter 2), it was hypothesized that the compounds' sorption to soil and plant was the major removal process based on recent findings (Beausse 2004; Loffler et al. 2005; ter Laak et al. 2006; Kinney et al. 2006; Chefetz et al. 2008; Stein et al. 2008; Kwon and Armbrust 2008). Therefore, in order to test the hypothesis, soil and plant samples were tested for the accumulation of the compounds, which is the purpose of this research.

At the end of the test period, soil and plant samples were collected to determine the concentration of the compounds contained within each. Soil samples were taken from the top (15 cm), a middle-depth (15-45 cm) and at the bottom (45-75 cm) layers of the soil. An auger was used to obtain soil samples, in triplicate, from the 15-cm top soil and at 30 cm intervals through the column height. Grass samples were collected using nitrile gloves and sterile cutter. Once collected, soil and plant samples were placed in sterile amber jars, capped and labeled properly. The soil samples were moved to the laboratory and dried under temperature less than 30°C to avoid any transformation of the organic compounds.

Sample Analysis

Soil and plant samples were moved to the laboratory where the dispersed Solid Phase Extraction (SPE) method followed by Liquid Chromatography and Mass Spectrometery (MS) were used to partition the target compounds from soil and plant samples.

1. Sample preparation

Dried soil samples were crushed to reach the maximum size of 0.2 mm. Plant samples were ground using a blender. Liquid-solid extraction followed by QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Lehotay 2011) was used for sample preparation. Stock solutions of internal standards, a mixture of acetaminophen-d4, trimethoprim-d9, carbamazepine-d10 and fluoxetine-d6, were prepared to monitor the analytical method efficiency. For soil samples, 16 mL of acetonitrile, 11 mL of nano-pure water, 1 mL of ammonia aqueous (20%) and 40 μL of internal standard solution were added to the test tube containing 20 g of soil. The test tube was shaken for 15 min at 250 rpm and then centrifuged at 3500 rpm for 15 min. The decant was added to the QuEChERS tube containing 6 g of anhydrous magnesium sulfate and 1.5 g of anhydrous sodium acetate. For plant samples, 5 g of plant tissue was mixed with ASE Prep DE (Dionex P/N 062819) to fill up an 11-mL extraction cell, and extracted by an

ASE 200 accelerated solvent extractor (ASE, Dionex Corporation, Sunnyvale, CA). Acetonitrile-water (1:1, v/v) solution was used as the extraction solvent. The extraction pressure and temperature were 1500 psi and 40 °C, respectively. The extracts from ASE were added to the QuEChERS tubes similar as the soil samples. The QuEChERS tubes were well shaken and centrifuged at 3500 rpm for 10 min to separate the acetonitrile and water layers. Aliquots of the upper acetonitrile layer were placed in a clean glass tube and evaporated to dryness using a gentle stream of nitrogen gas. The dry residues were reconstituted with 400 µL of 20% aqueous methanol, and the solution was filtered through a 0.45 µm HPLC filter before it was analyzed by LC-MS/MS.

2. Liquid Chromatography (LC)

A Thermo Fisher Scientific (San Jose, CA). Surveyor LC system consisting of a degasser, a quaternary LC pump, and an autosampler was used for the compound detection from the extracts. Separations were carried out in a Gemini NX-C18 analytical column (150 mm x 2.0 mm, 3 µm particle size), preceded by a SecurityGuard cartridge (4 mm x 2.0 mm, Gemini NX-C18). Both were from Phenomenex (Torrance, CA), and maintained at 40 ºC. The injection volume was 25 µL. The mobile phase was comprised of two solution mixtures; (A) 5% aqueous methanol with 5 mM ammonium formate and 0.05% formic acid and (B) 100% methanol with 5 mM ammonium formate and 0.05% formic acid. The LC gradient elution conditions are listed in Table 7.

Table 7. The liquid chromatography solvent mixing gradient program.

Time (min)	Flow (mL/min)	A (%)*	B (%)**
0	0.20	90	10
3	0.20	90	10
13	0.20	0	100
18	0.20	0	100
18.1	0.20	90	10
32	0.20	90	10

* Percent of solution mixture of solution A, consisting of 5% aqueous methanol with 5 mM ammonium formate and 0.05% formic acid.
** Percent of solution mixture of solution B, consisting of 100% methanol with 5 mM ammonium formate and 0.05% formic acid.

3. Mass spectrometry (MS)

Mass spectra were acquired using a LCQ Advantage ion trap mass spectrometer from Thermo Fisher Scientific (San Jose, CA). .Helium was the damping and collision gas for the ion trap while nitrogen served as sheath and auxiliary gases for the ion source. The mass spectrometer was automatically tuned in positive electrospray ionization (ESI) mode by pumping a neat standard solution (2 mg/L in 10% methanol with 5 mM ammonium formate and 0.05% formic acid) at a flow rate of 0.2 mL/min. Typical MS parameters included spray voltage, 4 kV; sheath gas flow rate, 48 arb ; auxiliary gas flow rate, 22 arb; capillary temperature, 250 ºC; capillary voltage, 11 V and tube lens offset, 25 V. The MS was

operated in the selected reaction monitoring (SRM) mode with ion transitions listed in Table 8. Raw data were collected and processed with Xcalibur 2.0.7 SP1 software provided by Thermo.

Table 8. The selected reaction monitoring (SRM) transitions and retention times for the target compounds and internal standards separated by mass spectrometery.

Compound	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (%)
Acetaminophen	4.26	152.1	110.0	36
Acetaminophen-d4	4.19	156.0	114.0	36
Trimethoprim	7.18	291.3	230.0	40
Trimethoprim-d9	6.87	300.0	234.0	40
Carbamazepine	16.67	237.1	194.1	38
Carbamazepine-d10	16.62	247.0	204.0	38
Fluoxetine	16.58	310.1	148.0	26
Fluoxetine-d6	16.52	316.0	154.0	26

Soil Dissipation Parameters

EPI (Estimation Program Interface) Suite software (US Environmental Protection Agency 2012) has been used to predict some of the parameters required for target pharmaceuticals fate and transport analyses. BIOWIN and KOCWIN sub-programs were of more interest in this research to predict the biodegradability and sorption affinity of the compounds to the soil, respectively. BIOWIN gives an estimate of the probability that the compound biodegrades in the environment and KOCWIN estimates the sorption coefficient (K_{oc}) to unit weight of organic carbon (OC) in soil. Two different Log K_{oc} values are given; one is based on a linear model with Log K_{ow} as the variable and the other is based on first-order molecular connectivity index (MCI) model shown in Equation 1. Log K_{oc} values could be used to determine the soil sorption coefficient K_d based on soil organic carbon content (Equation 2). When measured data are available on soil and solution (water) concentrations of the compound, k_d values can be calculated based on Equation 3.

$$\text{Log } K_{oc} = a * (\text{Log } K_{ow} \text{ (or MCI)}) + b \quad (1)$$

$$K_d = K_{oc} * (\text{fraction of soil organic carbon}) \quad (2)$$

$$K_d(\text{L/kg}) = \text{Soil Concentration (mg/kg)} / \text{Solution Concentration (mg/L)} \quad (3)$$

Data Analysis

Several comparisons have been performed in this study such as comparison of the compounds concentration in different column types and comparison of the concentrations at the different depths in each type of column. All statistical analyses were performed using SAS 9.3 software (SAS, 9.3). Student's t-test was used for comparison with triplicate data points. The significance level was considered 0.05 unless otherwise is stated in the results section.

Results and Discussions

Variations of Accumulation Along Soil Depth

The detected concentrations of PPCPs in plant and soil samples at different depth are presented in Table 9. Acetaminophen and fluoxetine were not detected in any samples while consistent detection of carbamazepine and trimethoprim was obtained. Comparing analyses results of PPCP concentrations in soil samples collected from different column depths (Table 9), carbamazepine was found with the highest concentration (45 ± 20 ng/g) in the middle-depth soil of the open columns whereas trimethoprim in top layer soil of the open columns had the highest concentration (1.6 ± 0.2 ng/g).

Acetaminophen is a compound determined to have very low affinity for sorption to different types of soils at natural pH levels (Lorphensri et al. 2006), which is explained by looking at its K_{ow} and pka values. It has been observed to decrease in concentration in the growth media of plants (Bartha et al. 2010). Bartha et al. (2010) suggested the presence of some unknown abiotic process may cause acetaminophen degradation in soil which is independent from plant existence. The probability for acetaminophen biodegradation is much higher than other target compounds based on BIOWIN estimations (US EPA 2012) where fluoxetine has the lowest probability for biodegradation. Fluoxetine has been considered persistent in biosolid amended soil (Walters et al. 2010; Redshaw et al. 2008) and had low biodegradation in soil, similar to carbamazepine (Monteiro and Boxall 2009; Dougherty 2011; Carballa et al. 2006). Kwon and Armbrust (2006) reported fluoxetine to be a very low biodegradable and photodegradable compound, although sorption to the sediments was dominantly observed, which is consistent with the results by Kinney et al. (2006). Fluoxetine is mainly present in its cationic form in soil pH of this study and will bind to the negatively charged clay surfaces; also its high Log K_{ow} value will cause higher sorption to organic matter.

A year of irrigation has resulted in the target compounds transfer to the bottom of the columns and thus leach out the bottom of the systems. In the grass and covered columns, trimethoprim was detected at higher concentrations in the bottom-depth of the soil column compared to the concentrations in the upper-depths of the columns ($P<0.15$) (Figure 6). However, carbamazepine concentrations in the top and middle-depth soil samples were higher than at the bottom-depth of soil with the significance level < 0.15 (Figure 7). Thus concentration of carbamazepine was highest at the middle-depth sampling point for grassed and covered columns which is consistent with the results from previous studies on watershed influenced by septic tank effluent (Dougherty 2011) and soil below a leaking trunk sewer (Ellis 2006; Wolf et al. 2004). Since this pattern was not observed for covered columns, it could be concluded that the plant activity and photodegradation may affect the top soil carbamazepine degradation. No significant pattern was seen for trimethoprim soil concentration along column depth.

The differences between trimethoprim and carbamazepine transport behavior may be a result of their physiochemical properties. Higher water solubility and lower soil sorption affinity, which is defined by K_{oc} could be reasons for trimethoprim quicker transport through the soil depth. In the previous chapter, the annual percolation rate of trimethoprim to the deeper soil, thus to groundwater has been, generally, determined to be higher than carbamazepine. From Table 9, it was shown that carbamazepine was detected in plant and soil at higher concentrations than trimethoprim. Transport of trimethoprim in open columns shows different pattern relative to other columns. The high accumulation of the compound at the

top layer of the soil might be due to quick evaporation during warm seasons, so the transport of the compound along with water was weakened.

Table 9: Dry weight concentration of the target compounds in the soil and plant samples (values are Mean ± Standard Deviation).

Compound	Plant	Top soil	Middle soil	Bottom soil	BCF*
Grass columns					
Acetaminophen	ND**	ND	ND	ND	NA
Carbamazepine	577 ± 61	24 ± 6	29 ± 9	18 ± 4	24.4
Trimethoprim	93 ± 6	0.7 ± 0.2	0.9 ± 0.3	1.1 ± 0.2	138
Fluoxetine	ND	ND	ND	ND	NA
Covered columns					
Acetaminophen	NA***	ND	ND	ND	NA
Carbamazepine	NA	33 ± 17	29 ± 4	19 ± 7	NA
Trimethoprim	NA	0.8 ± 0.2	0.7 ± 0.7	1.4 ± 2	NA
Fluoxetine	NA	ND	ND	ND	NA
Open columns					
Acetaminophen	NA	ND	ND	ND	NA
Carbamazepine	NA	44 ± 14	45 ± 20	35 ± 17	NA
Trimethoprim	NA	1.6 ± 0.2	1.5 ± 1	0.8 ± 0.7	NA
Fluoxetine	NA	ND	ND	ND	NA

* Bioconcentration Factor.

** Not Detected.

*** Not Applicable.

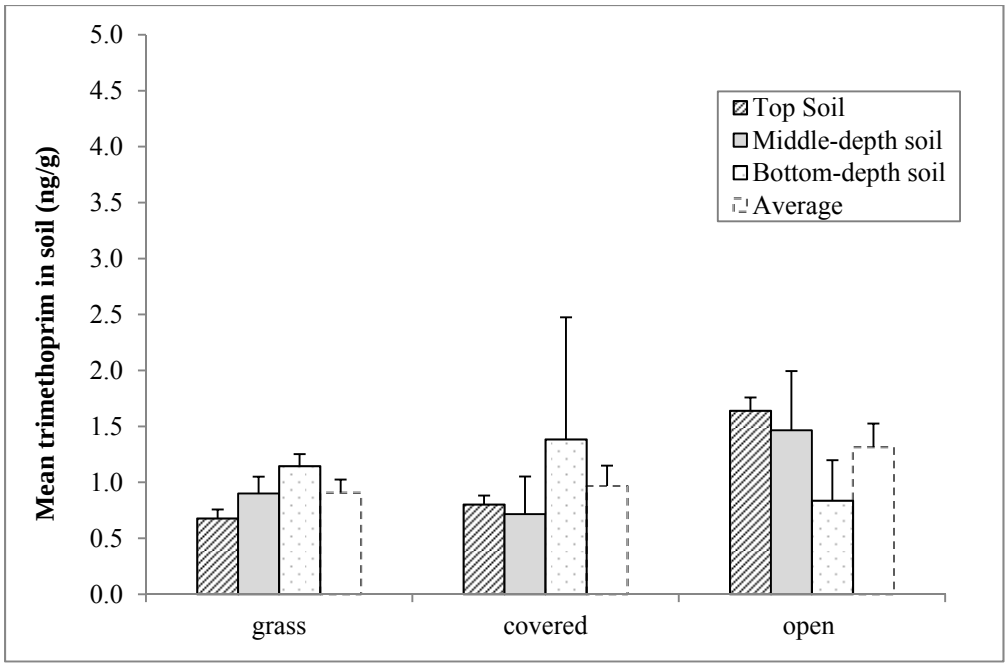


Figure 6: Comparison of trimethoprim concentration (mean ± standard deviation) at different soil depths and different column types.

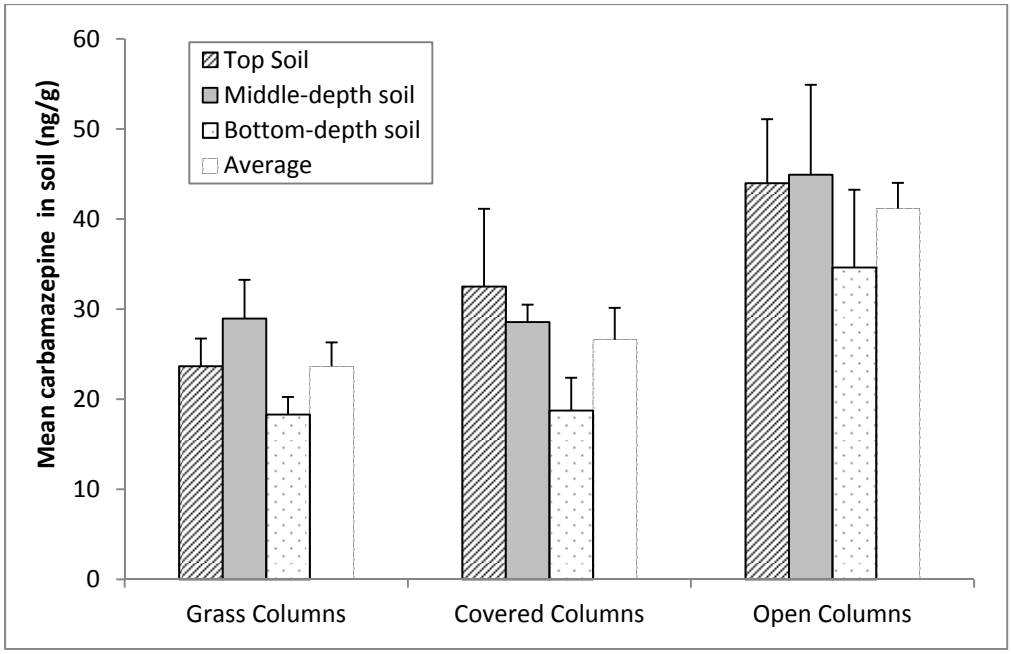


Figure 7: Comparison of carbamazepine concentration (mean ± standard deviation) at different soil depths and different column types.

Variations of Accumulation Among Different Column Types

Carbamazepine and trimethoprim accumulated amounts in top soil of the grass columns, presented in Figures 6 and 7, are lower than the accumulated amount in the top soil of the covered columns, which is lower than the accumulated amount in analogous samples from open columns with the significance of less than 0.2. This pattern implies that the presence of the plant could be a factor to consider for pharmaceuticals removal. The reason for higher concentrations in open columns is the rate of water application, which was higher for grass and open columns to satisfy the evapotranspiration, therefore a greater mass of pharmaceuticals were applied to these columns compared to the covered ones.

Carbamazepine and trimethoprim translocated to the plant tissues with bioconcentration factors (BCF) of 24.4 and 138, respectively. The bioconcentration factor is calculated by dividing the dry weight plant concentration of the pharmaceutical over the dry weight soil concentration of the specific compound. The mean accumulated amount of carbamazepine and trimethoprim in grass samples per dry weight are significantly ($P<0.01$) higher than the mean accumulated per dry weight in the soil samples, which results in relatively high bioconcentration factors for both compounds compared to previous studies. Shenker et al. (2011) reported the BCF for carbamazepine uptaken by cucumbers from different growth media to be at the range of 1.5 – 18, which was higher than BCF values determined for carbamazepine uptake by ryegrass, Lolium perenne, (Winker et al. 2010), cabbage, Brassica rapa var. pekinensis, (Herklotz et al. 2010), lettuce and carrot (Boxall et al. 2006), soybean, Glycine max (L.) Merr. (Wu et al. 2010) and several edible crops (Eggen and Lillo 2012). The BCF values calculated from models developed based on compound hydrophobicity properties like K_{ow} (Trapp 2009; Calderón-Preciado 2011) gives a much lower value than what was obtained from our experiment. Karnjanapiboonwong et al. (2011) investigated the BCF values for triclosan and an estradiol by pinto beans,(Phaseolusvulgaris), and BCF values were much higher for sand growth media comparing to soil. Therefore, other than compound hydrophobicity, soil and plant characteristics are also important for BCF calculations. The higher the K_{ow} of the compound, the more affinity for sorption to the soil, so carbamazepine have been accumulated to soil much more than trimethoprim. Even though the carbamazepine concentration in the plant is higher than trimethoprim, the amount sorbed to the soil caused a higher BCF for trimethoprim. Thus plant uptake of the compound is restricted due to the sorption to soil and organic matter. The dissociation constant, Pka, of the compound is another factor affecting transport of the chemicals to the plant tissue. For instance carbamazepine is a very weak acid and ionized at a very low degree at the typical west Texas soil pH. Trimethoprim is an ionizable compound in the same soil pH and will be in equilibrium with its ionized form. The Ionization of the chemical have been discovered to decrease its potential for plant uptake in hydroponic conditions because of the lower K_{ow} and lipophilic uptake for the ionized form (Trapp 2009). In the case of this study the combination of the soil and plant tends to lower BCF for the compound with a higher K_{ow} value.

Although the bioconcentration factors are high, the amount of plant harvested is small; therefore the total chemical mass removed by the plant is minimal comparing to the PPCP application rate. The total mass of plant material have been removed from the columns throughout the year was between 1300 – 12000 mg, while the amount extracted from soil and plant lies in the range of 0.05 – 2 mg, which is less than 1% of the mass applied. However, some portions of the pharmaceuticals are released to the subsections of the soil or groundwater and that amount is also less than 5% of the applied mass. Therefore, the main removal procedure in the soil columns could be the biodegradation by soil microbes, photodegradation, or transformation in the plant. This issue will raise the concern of the presence of the metabolites in the soil, plant or groundwater, however it is not assessed in this research.

Summary and Conclusions

This research has focused on a group of pharmaceuticals, including acetaminophen, carbamazepine, trimethoprim and fluoxetine emitted from onsite wastewater treatment systems and their fate and transport at a pilot scale. Most wastewater effluents assessed so far are carrying trace levels of these contaminants which would eventually transport to groundwater after land application of the effluent, and thus it is essential to determine the parameters affecting the fate of these chemicals. In locations where groundwater will be used as a source for drinking water, presence of these chemicals in water may poses unexpected further impacts. The pharmaceutical removal capacity of three types of wastewater land application systems including vegetated lawns, simulated unvegetated land covered from sun and bare lands was examined in this study to investigate the effect of grass and solar radiation in removal capacity of the land application systems. The leachate water and the soil and plant were collected and tested for pharmaceuticals contamination. A summary of major outcomes of this study follow.

Transport of Pharmaceuticals in Soil Following Onsite Wastewater Effluent Application

- Although all tested pharmaceuticals were detected in leachates of all columns at least once during the testing period and at the ranges of low ng/l to low µg/l of concentrations, removal rates were more than 95% in all conditions suggesting that the soil itself plays the major role in the removal process.
- The effect of photodegradation and removal by the grass was not significant in the removal rates of the four selected pharmaceuticals.
- The highest mean detected leachate concentration was observed for fluoxetine at 600 ng/l in the month of July while this compound showed the lowest frequency of detection.
- Relevant to their characteristics, carbamazepine and trimethoprim were detected more frequently and at higher concentrations than acetaminophen and fluoxetine in more than 95% of the events.
- The final evaluation of the annual removed mass is indicating that the grass columns had removed 30% and 15% more pharmaceuticals mass rather than covered and open columns, respectively. The results of this study suggest that vegetated lawns can maintain the required capacity for removing pharmaceuticals even at the times they are irrigated more than other treatment systems to maintain plant higher evapotranspiration.

Pharmaceuticals Accumulation in Soil and Plant Following Wastewater Land application

- Carbamazepine and trimethoprim were detected in soil and plant tissue. The higher concentrations were obtained for carbamazepine while a higher bioconcentration factor (BCF) was calculated for trimethoprim.
- Based on the results of this study the plant BCF does not have a positive relation with the Log K_{ow} value of the compound which is different from previous studies. This may suggest that the combination of soil organic matter and plant lipid content is also an important factor in defining the BCF for a long-term scale calculation.
- The presence of plant resulted in lower top soil pharmaceuticals contamination however, the difference was not significant at the 95% confidence interval level.
- Concentration of carbamazepine was highest at the middle-depth sampling point within the soil column for grassed and covered columns, which is consistent with the results from previous studies. Since this pattern was not observed for covered columns, it could be concluded that the plant activity and some photodegradation may affect the top soil contaminant degradation. No significant pattern was seen for trimethoprim.
- The mass balance analysis shows that less than 10% of the mass of compound applied to the soil is found in soil, plant and leached water samples, therefore other transformation pathways such as biodegradation in soil or plant are likely responsible for the loss of these compounds. These pathways may result in metabolites in the environment which will be a concern if the metabolites are toxic.

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Appendix (Original Data)

Concentrations of the Selected Pharmaceuticals in the Leachate of the Columns (ng/l) for Each Month of the Test Period

Jan

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	ND	ND	ND	ND	ND	ND	ND	ND	ND	250
Trimethoprim	ND	ND	ND	ND	ND	ND	ND	ND	ND	140000
Carbamazepine	ND	ND	ND	ND	ND	0.70	2.0	4.6	ND	25000
Fluoxetine	ND	ND	ND	ND	ND	ND	ND	ND	ND	120000
Water Volume Outlet (ml)	490	1640	157	500	4010	3870	330	110	1275	
Water Volume Inlet (ml)	13500	13500	13500	12000	12000	12000	12000	12000	12000	

Feb

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	32	13	4.2	ND	ND	ND	ND	41	540	84000
Trimethoprim	130	160	28	7.6	8.8	16	0.35	120	1200	180000
Carbamazepine	80	4.5	2.6	1.7	0.55	1.6	1.4	60	750	32000
Fluoxetine	ND	ND	ND	ND	ND	ND	ND	ND	Detected	70000
Water Volume Outlet (ml)	6050	5250	5500	4020	6070	4030	3520	900	1480	
Water Volume Inlet (ml)	13500	13500	13500	12000	12000	12000	12000	12000	12000	

Mar

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	9.3	10	37	4.5	5.0	4.6	ND	4.9	ND	230
Trimethoprim	110	130	270	37	14	23	11	23	10	100000
Carbamazepine	15	4.9	25	1.2	2.9	3.9	1.9	4.4	1.3	60000
Fluoxetine	Detected	ND	_	ND	ND	ND	ND	ND	ND	50000
Water Volume Outlet (ml)	1600	850	650	1800	2200	3450	1850	1000	1450	
Water Volume Inlet (ml)	6000	5950	6920	6058	5630	5845	6060	6110	6170	

Apr

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	NW	NW	NW	ND	ND	ND	NW	260	10	660
Trimethoprim	NW	NW	NW	7.0	6.8	39	NW	88	200	78000
Carbamazepine	NW	NW	NW	3.5	2.5	15	NW	50	160	87000
Fluoxetine	NW	NW	NW	ND	ND	ND	NW	Detected	Detected	42000
Water Volume Outlet (ml)	10	10	10	2400	3100	2800	60	550	850	
Water Volume Inlet (ml)	6380	6565	6140	6515	6550	5770	6030	6380	6600	

May

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	NW	NW	NW	ND	50	ND	NW	ND	NW	730
Trimethoprim	NW	NW	NW	36	91	2.5	NW	1.5	NW	140000
Carbamazepine	NW	NW	NW	17	32	7.0	NW	3.0	NW	73000
Fluoxetine	NW	NW	NW	ND	ND	ND	NW	ND	NW	35000
Water Volume Outlet (ml)	10	10	10	3040	3010	2850	10	1540	10	
Water Volume Inlet (ml)	6590	6490	6730	6430	6490	6420	6090	6205	6010	

June

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	NW	NW	NW	5.9	10	NW	NW	NW	NW	130
Trimethoprim	NW	NW	NW	27	33	NW	NW	NW	NW	170000
Carbamazepine	NW	NW	NW	8.9	10	NW	NW	NW	NW	75000
Fluoxetine	NW	NW	NW	ND	ND	NW	NW	NW	NW	25000
Water Volume Outlet (ml)	0	0	0	2320	2340	0	0	0	0	
Water Volume Inlet (ml)	7750	7750	7750	6200	6200	6200	6200	6200	6200	

July

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	NW	NW	NW	ND	ND	ND	NW	NW	NW	1300
Trimethoprim	NW	NW	NW	47	12	13	NW	NW	NW	210000
Carbamazepine	NW	NW	NW	13	4.8	9.4	NW	NW	NW	100000
Fluoxetine	NW	NW	NW	830	968	ND	NW	NW	NW	64000
Water Volume Outlet (ml)	0	0	0	2750	2200	1350	0	0	0	
Water Volume Inlet (ml)	11600	11600	11600	5220	5220	5220	7250	7250	7250	

Aug

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	ND	ND	ND	ND	ND	ND	NW	NW	NW	270
Trimethoprim	12	8.6	240	79	14	16	NW	NW	NW	190000
Carbamazepine	57	3.1	36	29	3.7	8.7	NW	NW	NW	87000
Fluoxetine	ND	ND	ND	ND	ND	ND	NW	NW	NW	40000
Water Volume Outlet (ml)	2120	2580	1930	2150	1180	1580	0	0	0	
Water Volume Inlet (ml)	17600	17600	17600	5120	5120	5120	11200	11200	11200	

Sep

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	NW	ND	NW	ND	ND	ND	ND	NW	ND	7100
Trimethoprim	NW	ND	NW	11	5.4	47	2.9	NW	1.9	110000
Carbamazepine	NW	160	NW	4.2	2.4	100	1.3	NW	ND	90000
Fluoxetine	NW	ND	NW	ND	ND	ND	ND	NW	ND	36000
Water Volume Outlet (ml)	4810	4680	5880	2430	1730	1820	3190	1890	1340	
Water Volume Inlet (ml)	15000	15000	15000	4800	4800	4800	13500	13500	13500	

Oct

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	ND	ND	ND	ND	ND	ND	ND	ND	ND	30000
Trimethoprim	ND	13	38	2.2	3.2	2.5	1.7	3.4	2.2	110000
Carbamazepine	6.2	91	74	<MDL	1.1	6.5	<MDL	0.65	<MDL	29000
Fluoxetine	ND	ND	ND	ND	ND	ND	ND	ND	ND	31000
Water Volume Outlet (ml)	6160	5840	4790	2820	3680	2640	3250	2830	3130	
Water Volume Inlet (ml)	12000	12000	12000	4800	4800	4800	12000	12000	12000	

Nov

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	ND	ND	ND	ND	ND	NW	ND	ND	ND	21000
Trimethoprim	ND	ND	67.798	11.272	8.084	NW	4.844	4.882	4.206	98000
Carbamazepine	4.616	70.724	200	1.5	<MDL	NW	1.818	1.098	<MDL	26000
Fluoxetine	ND	ND	ND	ND	ND	NW	ND	ND	ND	64000
Water Volume Outlet (ml)	5080	5340	4500	2280	2090	0	3630	2400	3210	
Water Volume Inlet (ml)	9000	9000	9000	4500	4500	4500	9000	8400	8400	

Dec

ng/l	Col # 1	Col # 2	Col # 3	Col # 4	Col # 5	Col # 6	Col # 7	Col # 8	Col # 9	Influent
Acetaminophen	ND	ND	ND	ND	ND	ND	ND	ND	ND	79.438
Trimethoprim	ND	ND	5.482	10.392	4.124	3.59	12.672	7.758	6.806	41000
Carbamazepine	5.396	56.298	47.764	9.506	0.926	0.964	3.454	2.222	12.822	16000
Fluoxetine	ND	ND	ND	240	ND	ND	ND	ND	ND	75000
Water Volume Outlet (ml)	5200	5080	4250	3000	3050	1870	3370	42200	3020	
Water Volume Inlet (ml)	7500	7500	7500	4500	4500	4500	7500	6700	7000	

ND : Not Detected.
NW: No water.
Col # 1, 2, 3 = Grassed columns (Replicates).
Col # 4, 5, 6 = Covered Columns (Reps).
Col # 7, 8, 9 = Open Columns (Reps).

Soil and Plant Data

Sample ID	Carbamazepine (ng/g)	Trimethoprim (ng/g)
C1B*	16.41757	1.23
C1M	19.33025	0.6
C1T	23.18689	0.733827
C2B	22.78314	1.305
C2M	35.85496	1.2
C2T	17.72915	0.5
C3B	15.67983	0.9
C3M	31.67085	0.9
C3T	30.0409	0.8
C4B	27.13336	3.899708
C4M	33.03438	0.770256
C4T	29.62376	0.931264
C5B	14.4714	0
C5M	26.52272	0.017501
C5T	51.02704	0.625454
C6B	14.61568	0.252415
C6M	26.10638	1.358736
C6T	16.86474	0.85
C7B	14.97015	0
C7M	22.34004	0.782057
C7T	44.00911	7.069213
C8B	47.52633	1.276941
C8M	52.04168	2.684342

C8T	23.29669	0.515616
C9B	41.33435	1.230274
C9M	60.37098	0.92872
C9T	64.65555	2.765
G1**	518.6949	97.26574
G2	640	89.45829
G3	571.4878	167.0595

* Column # and depth where B is bottom depth, M is middle depth and T is the top soil samples.

** Grass samples from column number.