

# **PHARMACEUTICAL COMPOUNDS IN LAND-APPLIED SLUDGE AND PLANT UPTAKE: A REVIEW**

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Submitted to the Graduate Faculty of  
North Carolina State University  
in partial fulfillment of the  
requirements for the Degree of

Master of Environmental Assessment

Raleigh, North Carolina

2015

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## **Abstract**

Taylor-Smith, Aana. Master of Environmental Assessment. Pharmaceutical compounds in land-applied sludge and plant uptake: A review.

In the fields of environmental science and wastewater treatment, pharmaceutical products are gaining recognition as potential contaminants of concern. Pharmaceuticals have been found to pass through conventional wastewater treatment facilities and enter surface waters, which has spurred research into ecotoxicological effects in surface waters. Less is known about pharmaceuticals in sludge from wastewater treatment. Pharmaceuticals and pharmaceutical residues present in sludge could have significant consequences due to land application of sludge on agricultural fields, where chemicals may partition into soils and be taken up by plants. The effects of uptake by plants are not yet fully understood, but some pharmaceutical compounds have been detected in plant tissues.

The objective of this study is to review what is currently known about pharmaceuticals, their transport through conventional wastewater treatment facilities, their detection in sludge, their pathways through soils and plants, and the consequences of pharmaceuticals in sludge to human and ecological health. Current efforts and strategies to prevent the release of pharmaceuticals to the environment are also reviewed. These topics were researched in a review of recent scientific literature from 79 peer-reviewed journals and government documents, 47 of which are cited in this review.

## Biography

Aana Taylor-Smith graduated in 2011 from the University of North Carolina at Wilmington (UNCW) with two Bachelor of Science degrees in Biology and Environmental Science. While at UNCW, Aana completed an independent research project on the effects of riparian vegetation on water quality in urban streams in conjunction with the City of Greensboro municipal government and Dr. Brian Arbogast.

Aana worked with the State of North Carolina Department of Environment and Natural Resources for two years as an Environmental Specialist, and now works for the Tennessee Department of Environment and Conservation as an Environmental Scientist. The Environmental Assessment (EA) program at North Carolina State University has provided her with an excellent way to advance her knowledge and foundation in environmental science. Aana wished to pursue the EA degree in order to better understand human-environmental interactions and help protect our environment for future generations.

## Acknowledgements

I would like to extend my sincere gratitude to Dr. Elizabeth Nichols for supporting my ideas and guiding my way throughout this project. You have always been available for assistance if I needed help, and I couldn't have done it without you. I would also like to thank my advisor, Dr. Linda Taylor, for keeping me on track throughout the EA program, as well as all of the NC State professors I have had the privilege of working with over the past few years.

I would also like to thank my supervisors and co-workers in North Carolina, especially Corey Basinger and Matthew Gantt, for supporting my work and answering my questions about environmental science. And finally, huge thanks to my family and friends for always being there for me and appreciating my work in environmental science. Go Wolfpack!

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## Introduction

An emerging concern in environmental science is the presence of pharmaceutical residues in waste streams such as wastewater and sewage sludge. These chemicals are released as a result of improper disposal, incomplete metabolism and release into municipal wastewater, industrial waste, and veterinary practices. Most of these sources are directed to wastewater treatment plants (WWTPs) for treatment. In the past, when attempting to control chemicals that affect water quality, regulators focused on pollutants like volatile organic chemicals (VOCs) and metals. They are controlled in the United States through a list of 126 Priority Pollutants established by the United States Environmental Protection Agency (USEPA) for which standards and analytical methods have been developed (USEPA 2014). However, none of these priority pollutants are pharmaceuticals.

Fortunately, over the past few years, improved analytical methods and techniques were developed to detect pharmaceuticals and pharmaceutical residues at the parts per billion (ppb) to parts per trillion (ppt) level (Jelić et al. 2011). Pharmaceuticals have been detected in environmental media as a result of discharge from WWTPs, industrial facilities, landfill leachate, and veterinary operations, among other sources. The main source of pharmaceuticals in the environment is WWTPs that treat municipal wastewater and discharge to surface waters (Fent et al. 2006); the primary contributors of pharmaceuticals in the waste stream are human use and excretion (Jasim et al. 2006). In most WWTP systems, pharmaceuticals can either degrade, adsorb to sludge, or be passed through in treated effluent to surface waters (Fent et al. 2006). This paper will focus on the portion of pharmaceuticals that adsorbs to sludge that is often removed and applied to land as a soil conditioner.

A wide range of drugs has been detected in wastewater influent, representing various classes of pharmaceuticals. The United States' per capita usage of pharmaceuticals in 2011 was 136.6 standard units (SU, defined as a single dosage unit) in both institutional and home environments (IMS 2012). Pharmaceutical classes detected in wastewater influent and effluent include analgesics, antibiotics,

anticonvulsants, antidepressants, anti-diabetics, beta-blockers, diuretics, histamine antagonists, lipid-regulating agents, and others (Jelić et al. 2011). This paper will focus on the presence of antibiotics, anticonvulsants, and non-steroidal anti-inflammatory drugs (NSAIDs) due to their ubiquitous use, differing chemical properties, and the attention given to those groups by scientific literature.

Almost all conventional WWTPs treat influent with an activated sludge system (Drillia et al. 2005). Many pharmaceutical compounds have a tendency to adsorb to sludge particles and biomass, removing them from effluent but making them a concern when that sludge is applied to land. After sludge is applied to land, compounds can adsorb to soil particles, run off in stormwater to surface water, or leach through soils to contaminate groundwater. A pharmaceutical's environmental fate is highly dependent on its physicochemical properties. Soil properties also play a major role in determining chemical fate in environmental media (Drillia et al. 2005).

Pharmaceuticals can be taken up from land applied sludge, manure, or soil into plants. Pharmaceutical compounds have been shown to be absorbed through plant roots from sediment or water and accumulate in leaves, stems, and fruits of edible plants like peas and cucumbers (Tanoue et al. 2012). Grain plants such as wheat, barley, and fescue have also been studied, as well as root vegetables like carrots (Eggen et al. 2011). Plant uptake is generally thought to be dependent on the properties of the pharmaceutical compound, the plant's physical characteristics, and soil properties (Tanoue et al. 2012).

Concerns have arisen with regards to the application of biosolids containing pharmaceutical compounds. The presence of pharmaceutical compounds in soils is in itself potentially hazardous. For example, antibiotic drugs could lead to antibiotic resistance in soil microbes or cause shifts in soil microbial activity (Clarke and Smith 2011). When pharmaceuticals are leached into groundwater or run off into surface water, they can present a danger to aquatic environments or affect drinking water

supplies. Finally, pharmaceuticals in the soil can be taken up into food crops and other plants. The potential effects of pharmaceutical compounds are wide-ranging and as yet poorly understood.

Many new techniques have focused on removing pharmaceutical compounds from the waste stream as a pre-treatment method at WWTPs. Membrane distillation with carbon nanotubes and ozonation have proven effective at pharmaceutical removal (Gethard et al. 2012, Rizzo et al. 2015, Jasim et al. 2006). Treatment of sewage sludge with fungi has also been tested. In one study, removal of pharmaceutical compounds with fungi also reduced toxicity of the sewage (Rodríguez-Rodríguez et al. 2011). This has important consequences for the land application of sludge and reduction of potential ecotoxicological effects.

## Methods

Between November 2014 and March 2015, scientific literature regarding pharmaceutical compounds in wastewater, sludge, soil, and plants was reviewed. The review focused on pharmaceuticals detected in WWTPs and sludge, fate and transport of pharmaceuticals in the environment, presence of pharmaceuticals in soils after application of sludge, presence of pharmaceuticals in crops grown in soils with biosolids, effects and consequences of those compounds entering the environment, and potential methods of remediation.

Keyword searches were conducted through the North Carolina State University library webpage (<http://www.lib.ncsu.edu>) and Google Scholar (<http://scholar.google.com>). Keywords included, but were not limited to, the following: *adsorption, agriculture, antibiotic, anticonvulsant, antiepileptic, anti-inflammatory, biosolids, detection, effluent, environmental impact, land application, NSAID, pass through, pharmaceuticals, plant uptake, removal, sludge, soil, wastewater treatment, and WWTP*. Additional literature sources were found by reviewing reference lists of selected peer-reviewed studies.

Publications used in this review included peer-reviewed scientific studies, review articles, government publications, and materials from non-governmental research organizations. Most documents were found online and have been referenced as such in the References section of this paper. In total, this study identified 79 publications relevant to the topics discussed; 47 were cited in the literature review. Scientific literature was selected without regard to international origin and no emphasis was placed on literature from a particular country or region. Selection criteria involved identifying whether studies contained information regarding the primary topics, whether the pharmaceutical groups of interest were discussed, and whether new information was presented.

## Results & Discussion

### Pharmaceuticals in the waste stream

Pharmaceuticals make their way into the domestic waste stream primarily through human use and excretion. These compounds can be excreted either as the unchanged original compound or as metabolites or conjugate acids – generally glucuronic or sulfuric acid (Gros et al. 2010). Conventional wastewater treatment practices, such as settling, aeration, coagulation, flocculation, filtration, and chlorination, have proven to be ineffective at removing pharmaceuticals and pharmaceutical residues from incoming wastewater (Uslu et al. 2011). The ability of WWTPs to remove pharmaceuticals is variable and depends largely on the type of wastewater treatment and type of pharmaceutical. One study found that removal rates of 73 pharmaceuticals ranged from 40-99% at seven Spanish WWTPs (Gros et al. 2010).

Data suggest that without advanced treatment methods beyond those present at conventional WWTPs, pharmaceutical compounds can be detected in all effluent discharge regardless of geographical location (Daughton 2003). Indeed, one study performed by the United States Geological Survey (USGS) found at least 1 of 95 pharmaceuticals and personal care products in 80% of 139 streams surveyed over 30 states between 1999 and 2000. Seventy-five percent of streams sampled contained more than one such chemical (Kolpin et al. 2002). That study provided a demonstrative overview of the ubiquitous nature of pharmaceutical compounds and their ability to bypass wastewater treatment.

This review focused on results for three major groups of pharmaceuticals: antibiotics, anticonvulsants, and NSAIDs. These major groups are ubiquitous in use and are frequently found in wastewater across the world. See Table 1, below, for more information on each group, as well as a list of studies included in this review with information on each group:

**Table 1.** Groups of pharmaceuticals in this review. Note that lists of examples are not all-inclusive.

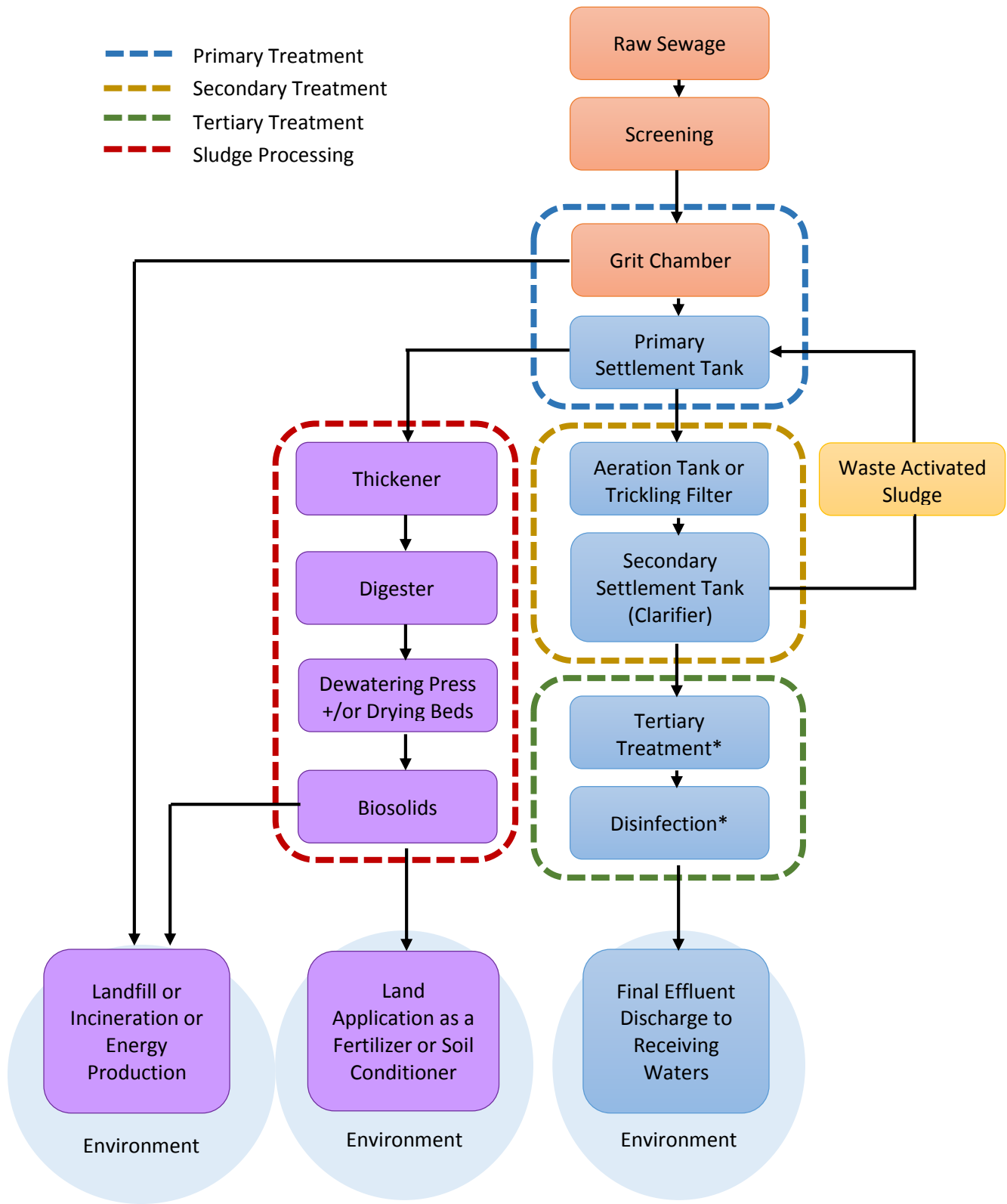
Group	Use	Examples	References
Antibiotics	Antimicrobial agents used to treat or prevent bacterial infections orally, topically, or intravenously. Can also be used as an anti-fungal in some cases. Used in human and veterinary medicine.	<p><b>Aminoglycosides:</b> Gentamicin, neomycin, streptomycin, etc.</p> <p><b>Cephalosporins:</b> Cefadroxil, cefaclor, ceftriaxone, cefepime, ceftobiprole, etc.</p> <p><b>Glycopeptides:</b> Telavancin, vancomycin, etc.</p> <p><b>Lincosamides:</b> Clindamycin, etc.</p> <p><b>Macrolides:</b> Clarithromycin, erythromycin, etc.</p> <p><b>Nitrofurans:</b> Furazolidone, nitrofurantoin, etc.</p> <p><b>Penicillins:</b> Amoxicillin, azlocillin, etc.</p> <p><b>Polypeptides:</b> Bacitracin, polymyxin B, etc.</p> <p><b>Quinolones/fluoroquinolones:</b> Ciprofloxacin, ofloxacin, temafloxacin, etc.</p> <p><b>Sulfonamides:</b> Sulfamethazine, Sulfamethoxazole, TMP-SMX, etc.</p> <p><b>Tetracyclines:</b> Doxycycline, tetracycline, etc.</p> <p><b>Others:</b> Chloramphenicol, clofazimine, rifampin, streptomycin, trimethoprim, etc.</p>	<p>Carvalho et al. 2014, Clarke and Smith 2011, Díaz-Cruz et al. 2009, Drillia et al. 2005, Eggen et al. 2011, Gros et al. 2010, Hörsing et al. 2011, Jasim et al. 2006, Jelić et al. 2011, Jones et al. 2014, Jones-Lepp and Stevens 2007, Kang et al. 2013, Kinney et al. 2006, Kolpin et al. 2002, Martín et al. 2015, Nieto et al. 2010, Pala-Ozkok et al. 2014, Rodríguez-Rodríguez et al. 2011, Rodríguez-Rodríguez et al. 2012, Sabourin et al. 2012, Tanoue et al. 2012, Thiele-Bruhn 2003, Topp et al. 2008, Uslu et al. 2011, Walters et al. 2010, C. Wu et al. 2012, X. Wu et al. 2012</p>
Anticonvulsants, anti-epileptics	Agents used in the treatment of seizures. Increasingly used to treat psychological or mood disorders like bipolar disorder and to treat neuropathic pain.	<p><b>Benzodiazepines:</b> Clonazepam, lorazepam, midazolam, etc.</p> <p><b>Carboxamides:</b> Carbamazepine, oxcarbazepine, etc.</p> <p><b>Oxazolinediones:</b> Ethadione, etc.</p> <p><b>Sulfonamides:</b> Acetazolamide, methazolamide, zonisamide, etc.</p> <p><b>Triazines:</b> Lamotrigine</p> <p><b>Others:</b> Parampanel, valnoctamide, etc.</p>	<p>Carvalho et al. 2014, Chefetz et al. 2008, Clarke and Smith 2011, Díaz-Cruz et al. 2009, Drillia et al. 2005, Fent et al. 2006, Gielen et al. 2009, Gros et al. 2010, Hörsing et al. 2011, Jasim et al. 2006, Jelić et al. 2011, Jones-Lepp and Stevens 2007, Kinney et al. 2006, Kolpin et al. 2002, Martín et al. 2015, Nieto et al. 2007, Nieto et al. 2010, Prosser et al. 2014, Rizzo et al. 2015, Rodríguez-Rodríguez et al. 2011, Rodríguez-Rodríguez et al. 2012, Sabourin et al. 2012, Tanoue et al. 2012, Ternes 1998, Topp et al. 2008, Uslu et al. 2011, Walters et al. 2010, C. Wu et al. 2012, X. Wu et al. 2012, Yu and Wu 2012</p>
NSAIDs	Nonsteroidal anti-inflammatory drugs. Used for analgesic, anti-inflammatory, and fever reducing effects.	<p><b>Acetic acids:</b> Diclofenac, indomethacin, etc.</p> <p><b>Anthranilic acids:</b> Mefenamic acid, etc.</p> <p><b>Enolic acids:</b> Meloxicam, piroxicam, etc.</p> <p><b>Propionic acids:</b> Fenoprofen, ibuprofen, ketoprofen, naproxen, etc.</p> <p><b>Salicylates:</b> Acetylsalicylic acid (aspirin), diflunisal, salicylic acid, etc.</p> <p><b>Others:</b> Clonixin, firocoxib</p>	<p>Carvalho et al. 2014, Chefetz et al. 2008, Clarke and Smith 2011, Díaz-Cruz et al. 2009, Fent et al. 2006, Gros et al. 2010, Gielen et al. 2009, Hörsing et al. 2011, Jasim et al. 2006, Jelić et al. 2011, Jones et al. 2006, Jones et al. 2014, Jones-Lepp and Stevens 2007, Kolpin et al. 2002, Martín et al. 2015, Nieto et al. 2007, Nieto et al. 2010, Rizzo et al. 2015, Rodríguez-Rodríguez et al. 2011, Rodríguez-Rodríguez et al. 2012, Sabourin et al. 2012, Tanoue et al. 2012, Ternes 1998, Topp et al. 2008, Uslu et al. 2011, Walters et al. 2010, X. Wu et al. 2012, Xu et al. 2009, Yu and Wu 2012</p>

### **Pharmaceuticals in sewage sludge**

As previously stated, most WWTPs use activated sludge technology to treat wastewater (Drillia et al. 2005). In this process, solid matter is first separated from the waste stream during preliminary treatment, then additional heavier particles are allowed to settle out by gravity in a sedimentation process referred to as primary treatment. Material removed at this phase is primary sludge and generally undergoes further treatment before being used as biosolids (UNEP DTIE 2002). Next, organic compounds are removed via microbial activity in secondary treatment, after which particles are allowed to settle out again to form secondary sludge. During secondary treatment, activated sludge removed from secondary sedimentation may be added back into the waste stream to allow for microorganisms used in secondary treatment to thrive (UNEP DTIE 2002). See Figure 1 for a depiction of the typical wastewater treatment process and generation of biosolids.

The amount of sludge removed at a WWTP can be estimated through calculations as demonstrated by Henze et al. (2002). For a WWTP with about two hours of settling time, 210 grams (g) of primary sludge per cubic meter ( $m^3$ ) of treated wastewater is removed, and 110-165  $g/m^3$  of secondary sludge is removed, depending on wastewater load at the WWTP (Hörsing et al. 2011). It has also been estimated that the average WWTP produces about 240 kilograms (kg) of dry solid material per million liters (l) of wastewater treated (Kinney et al. 2006). Sludge must be further treated before it is suitable for land application, as seen in Figure 1.

Fig. 1. Depiction of a typical WWTP process and biosolids generation. Adapted from UNEP DTIE 2002.



While various methodologies have been developed to analyze water for over 100 pharmaceutical compounds, the analysis of sewage sludge for pharmaceuticals has not advanced as quickly (Díaz-Cruz et al. 2009). Sewage sludge is a difficult matrix to analyze due to its complexity. Composition of sludge can vary based on inputs to the WWTP, and the sludge matrix often lacks uniformity. Furthermore, some components of sludge, such as surfactants or colloids added during the treatment process, can interfere with analytical methods (Díaz-Cruz et al. 2009). Commonly used methods for extracting pharmaceutical compounds from sewage sludge have included accelerated solvent extraction (ASE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), solid phase extraction (SPE), and ultrasonic extraction (USE) (Díaz-Cruz et al. 2009, Jelić et al. 2011, Nieto et al. 2009, Yu and Wu 2012). One study found that the most effective method of extraction was SPE (Díaz-Cruz et al. 2009), but other methods are routinely used. For separation and detection, common methods include gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), with tandem mass spectrometry (MS-MS) methods generally preferred for their increased analytical sensitivity (Díaz-Cruz et al. 2009). USEPA Method 1694 uses SPE with high performance liquid chromatography (HPLC) and MS-MS to detect pharmaceuticals in liquid and solid matrices (USEPA 2007).

Primary and secondary sludges have different consistencies, with primary sludge being described as similar to “wadding” in its appearance and secondary sludge like “instant coffee,” (Hörsing et al. 2011). The differences in process used to generate primary and secondary sludge, as well as physical differences, have led researchers to test and compare both types for the presence of pharmaceutical compounds. In a 2014 study, researchers found that the type of sludge did not have a significant effect on the concentration of trace pharmaceutical compounds detected, with the exception of the NSAID ibuprofen and the antibiotic erythromycin. Both of these pharmaceuticals were detected in higher concentrations in primary and mixed sludge. This difference was attributed to biodegradation

processes in the sludge rather than physicochemical properties such as  $K_{ow}$  (Jones et al. 2014). A 2011 study of 75 pharmaceutical compounds in sludge found that most compounds exhibited similar behavior towards primary and secondary sludge, but that ibuprofen again had a higher affinity for primary sludge (Hörsing et al. 2011). Finally, a study published in 2015 showed that NSAIDs and the anticonvulsant carbamazepine were found in higher concentrations in primary sludge, while antibiotics tended to be found more in secondary sludge (Martín et al. 2015). Differences in pH and temperature of sludge may also play a role in the concentration of pharmaceutical compounds (Hörsing et al. 2011).

Concentrations of pharmaceutical compounds found in sewage sludge vary, but some compounds have been detected in some amount in the majority of studies reviewed. Some of the most frequently studied antibiotics include ciprofloxacin, clarithromycin, erythromycin, ofloxacin, sulfamethazine, sulfamethoxazole, and trimethoprim. The most commonly studied anticonvulsant/anti-epileptic is carbamazepine, but some studies also focus on other anticonvulsants, particularly the benzodiazepines. Commonly studied NSAIDs include diclofenac, ibuprofen, ketoprofen, and naproxen. Table 2 provides ranges of the above-listed compounds found in sewage sludge in the studies reviewed for this paper.

**Table 2.** Ranges of pharmaceutical concentrations detected in sewage sludge.

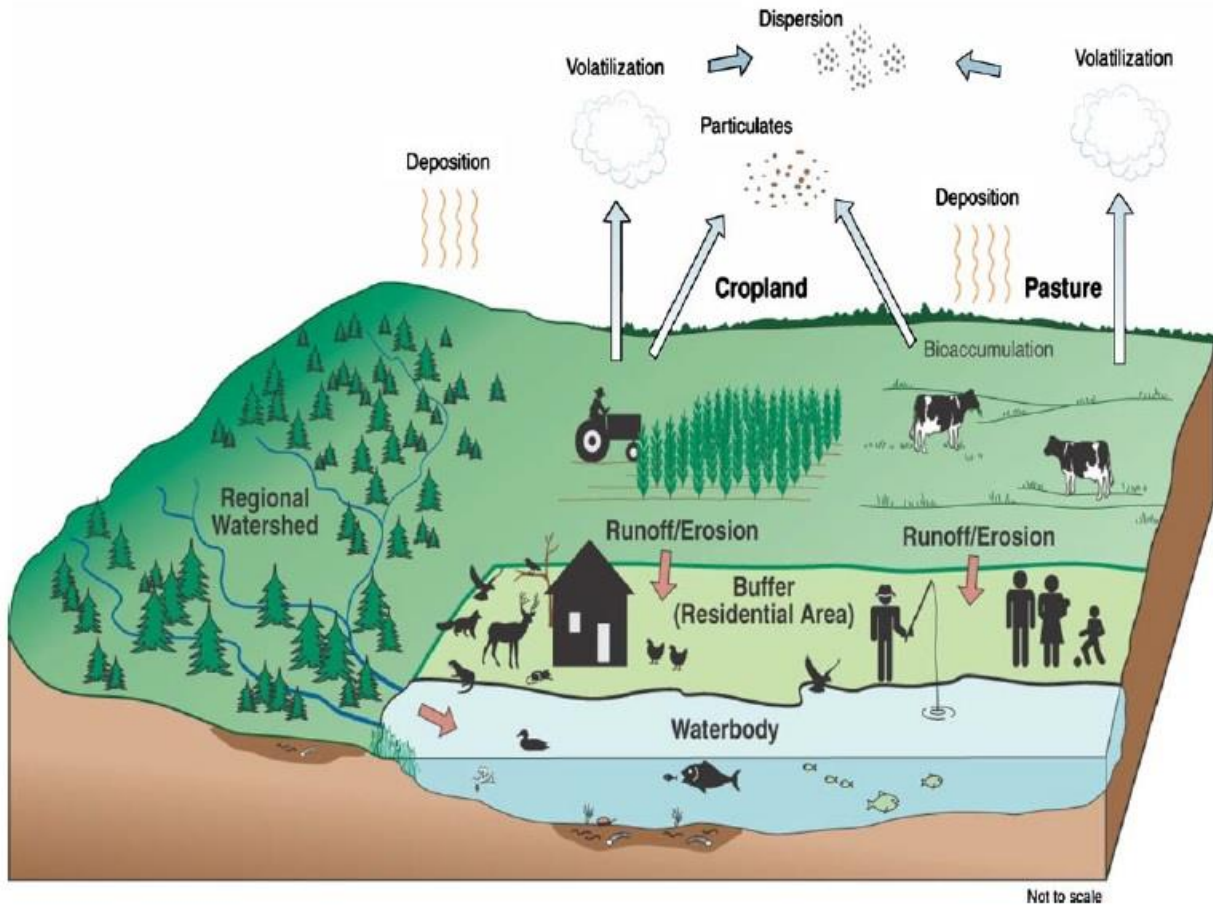
Group	Pharmaceutical Compound	Range Detected in Sewage Sludge ( $\mu\text{g}/\text{kg}$ )	References
Antibiotics	Ciprofloxacin	0.9-40,800	Clarke and Smith 2011, Díaz-Cruz et al. 2009, Jones-Lepp and Stevens 2007, Martín et al. 2015, Nieto et al. 2010
	Clarithromycin	0.3-67,000	Díaz-Cruz et al. 2009, Jelić et al. 2011, Jones-Lepp and Stevens 2007, Nieto et al. 2010
	Erythromycin	30-111	Jones et al. 2014, Jones-Lepp and Stevens 2007, Nieto et al. 2010
	Ofloxacin	0.1-58,100	Clarke and Smith 2011, Díaz-Cruz et al. 2009, Jones et al. 2014, Martín et al. 2015, Nieto et al. 2010
	Sulfamethazine	0.13-0.8	Díaz-Cruz et al. 2009, Jelić et al. 2011, Nieto et al. 2010
	Sulfamethoxazole	0.6-68,000	Díaz-Cruz et al. 2009, Jones-Lepp and Stevens 2007, Martín et al. 2015, Nieto et al. 2010
Anticonvulsants, anti-epileptics	Trimethoprim	0.6-133	Díaz-Cruz et al. 2009, Jelić et al. 2011, Martín et al. 2015
	Carbamazepine	4.7-1,200	Clarke and Smith 2011, Díaz-Cruz et al. 2009, Jones-Lepp and Stevens 2007, Kinney et al. 2006, Nieto et al. 2007, Nieto et al. 2010, Yu and Wu 2012
	Diclofenac	1.09-424.7	Díaz-Cruz et al. 2009, Jelić et al. 2011, Jones et al. 2014, Martín et al. 2015, Nieto et al. 2007, Nieto et al. 2010, Yu and Wu 2012
NSAIDs	Ibuprofen	6.34-548	Jelić et al. 2011, Jones et al. 2014, Martín et al. 2015, Nieto et al. 2007, Nieto et al. 2010, Yu and Wu 2012
	Ketoprofen	1.3-211	Jelić et al. 2011, Martín et al. 2015, Nieto et al. 2010, Yu and Wu 2012
	Naproxen	0.9-242	Jelić et al. 2011, Martín et al. 2015, Nieto et al. 2007, Nieto et al. 2010, Yu and Wu 2012

### **Environmental fate of pharmaceuticals in land applied sludge**

Around 50% of sewage sludge in the United States is applied to soils as a conditioning agent each year (Kinney et al. 2006). Similar percentages can be seen around the world, with around 53% of sewage sludge being land applied in the European Union (Martín et al. 2015), 40% in Canada (Topp et al. 2008), and around 14% in Japan (Tanoue et al. 2012). This number is likely to grow as demand for food production increases and phosphorus supplies decrease (Martín et al. 2015). Prior to being applied to soil as a soil conditioner, sewage sludge is treated through means such as thickening, stabilization, conditioning, and de-watering. The aim of these treatments is to reduce pollutants of concern and pathogens while optimizing the sludge's fertilizing and conditioning properties (Jones-Lepp and Stevens 2007). However, these practices do not eliminate all pharmaceuticals, and many compounds are present after sludge is applied to soils.

In 1993, the USEPA conducted a risk assessment for compounds found in sewage sludge, primarily metals and other priority pollutants, which demonstrated possible pathways for contaminants from sewage sludge to the environment (Jones-Lepp 2007). Figure 2 demonstrates that compounds contained in sewage sludge can adsorb to soil particles, leach into groundwater, run off in stormwater to contaminate surface waters, or volatilize into the air. Currently, no evidence suggests that pharmaceuticals partition into the air in significant amounts.

Fig. 2: Potential environmental fates of compounds in sewage sludge (Jones-Lepp 2007, p. 1180).



A general model for the behavior of pharmaceutical compounds in soils is difficult to conceptualize due to the wide variations in pharmaceutical compounds in sludge, physicochemical differences in pharmaceuticals, differences in soil types and organic content, and climactic differences. However, Drillia et al. (2005) found that a pharmaceutical's tendency to move through soil is correlated with its tendency to sorb to soil particles. The propensity to sorb to soil particles is dependent on a compound's physicochemical properties. For example, in one study, the antibiotic ofloxacin sorbed most strongly to soil particles, while diclofenac, carbamazepine, and sulfamethoxazole showed weaker sorption, respectively. This indicates that ofloxacin is more likely to remain tightly bound to soil particles and is less bioavailable, while the opposite is true for sulfamethoxazole, which is more likely to leach off

into ground water or surface water (Drillia et al. 2005). Table 3 shows the physicochemical properties of these compounds.

The difference in sorption patterns of the fluorquinolone ofloxacin and the sulfonamide sulfamethoxazole demonstrates the importance of a compound's individual properties to the analysis of its behavior in environmental matrices. Although both of these compounds belong to the same therapeutic group, antibiotics, they have different physicochemical properties, such as  $\log K_{OW}$  and  $pK_a$  values. The  $\log K_{OW}$ , or log of the octanol-water partitioning coefficient, represents a compound's propensity to partition into either non-polar or polar mediums. Compounds with a high  $\log K_{OW}$  are typically hydrophobic, with a low water solubility, while those with a low or negative  $\log K_{OW}$  are typically hydrophilic with a higher water solubility. The  $pK_a$ , or acid dissociation constant, represents a compound's ionization state. This is important because chemicals with a  $pK_a$  lower than neutral, such as NSAIDs like ibuprofen, diclofenac, and ketoprofen, will most likely be ions in the environment, making them more likely to dissolve into water than adsorb to solids (Fent et al. 2006).

Fluorquinolones typically have a much higher soil-organic content partitioning coefficient ( $K_{OC}$ ) (Thiele-Bruhn 2003), which is calculated from the  $K_{OW}$  and represents a compound's likelihood to bind to soil. Depending on the  $K_{OC}$ , which is often expressed as  $\log K_{OC}$ , a compound may preferentially bind to organic matter or soil particles, and a higher  $\log K_{OC}$  generally indicates lower water solubility. Organic content varies widely between soil types, ranging from 99-390 mg/g in one study alone (Kinney et al. 2006). To further complicate matters, a compound's tendency to bind to soil also depends on clay content, pH, and ionization state. The  $\log K_{OC}$  varies between soil types (Drillia et al. 2009); however,  $\log K_{OC}$  remains a useful indicator when determining a compound's behavior in the environment. It is important to note that the  $\log K_{OW}$  has a linear relationship with  $\log K_{OC}$ , and can therefore be used to predict a compound's behavior similarly to the  $\log K_{OC}$  when experimental data are unavailable for the  $\log K_{OC}$  (USEPA 1996).

Another factor in the environmental fate of pharmaceutical compounds is regional climate patterns. High intensity rain events cause a greater amount of desorption of pharmaceutical compounds from soil particles, leading to more pharmaceuticals recovered in leachate (Drillia et al. 2009). This suggests that in areas with large amounts of rainfall, pharmaceuticals may be found more in the aqueous phase, whether in groundwater or surface water, rather than adsorbed to soils.

Degradation of pharmaceutical compounds is an important method of removal from the environment. Anaerobic processes, such as photodegradation and hydrolysis often break pharmaceuticals down into their component parts (Thiele-Bruhn 2003). Microbial biodegradation can also occur, in which pharmaceutical compounds are metabolized or broken down by soil microbes and removed from the environment. NSAIDs such as ibuprofen and naproxen have been shown to be more readily biodegraded than other pharmaceuticals. Carbamazepine, in particular, was most resistant to microbial biodegradation and thus was detected more frequently in leachate from soils (Gielen et al. 2009). However, a higher initial concentration of pharmaceutical compounds has shown to decrease microbial degradation (Xu et al. 2009).

Despite the aforementioned difficulties in predicting a pharmaceutical compound's environmental fate, some general trends can be seen in scientific literature. Several types of antibiotics, especially fluoroquinolones, sulfonamides, and tetracyclines, may be removed through biodegradation as well as photodegradation (Thiele-Bruhn 2003). However, quinolone antibiotics have been shown to have half-lives in soils of 1,000 days or more due to their sorption to organic materials in soil. Tetracyclines also have longer half-lives than might be predicted, sometimes around 500 days (Walters et al. 2010). This could be attributable to other components in land applied sludge that may inhibit or interfere with microbial activity. Additionally, some biodegradation reactions may be reversible, and are often inhibited by the compound's binding to soil particles. Altogether, antibiotics have been found to

remain in soils longer than other types of pharmaceutical compounds, though this depends on the soil type (Thiele-Bruhn 2003).

The anticonvulsant carbamazepine is relatively resistant to biodegradation and is very commonly found in sewage sludge, making it more likely to accumulate in soils as it is reintroduced with each application (Kinney et al. 2006). One study found that carbamazepine's half-life in soils was around 495 days, which was significantly higher than the predicted 75 days (Walters et al. 2010). This may be due to the compound's tendency to sorb more strongly to soil particles with high organic content, which was described by Gielen et al. (2009). Interestingly, in some studies, carbamazepine was detected in greater quantities in soils than in the sludge applied to those soils. It has been hypothesized that carbamazepine conjugates that form in the human body are cleaved in aerobic soils, releasing more of the parent compound (Gielen et al. 2009). This factor may also contribute to the concentration of carbamazepine being higher than expected in soils. Despite its apparent abundance in soils, one study found that carbamazepine was found in greater quantities in surface and groundwater than sulfamethoxazole or diclofenac. Carbamazepine has an average tendency to bind to soils compared to other pharmaceuticals and is found both in the soil and aqueous matrices (Drillia et al. 2005).

Some NSAIDs, like ketoprofen, have been shown to adsorb more strongly to soils containing higher amounts of organic material, but not those with lower organic content. Others, like diclofenac and ibuprofen, have been shown to adsorb weakly to soil particles in general, making them more likely to leach out of soils and into groundwater (Xu et al. 2009). Runoff into surface water is a significant fate for NSAIDs, with ibuprofen and naproxen shown to partition rapidly into stormwater runoff after land application of biosolids (Topp et al. 2008). Another study found that ibuprofen and naproxen were not found in high concentrations in leachate from soils because they were likely degraded by microbial activity in the soil (Gielen et al. 2009). Indeed, ibuprofen, naproxen, and diclofenac were shown to have soil half-lives of around 6, 17, and 20 days, respectively (Xu et al. 2009), far lower than the half-lives for

antibiotics and anticonvulsants studied by Walters et al. (2010). With allowances made for differences in chemical structure, NSAIDs seem less likely to concentrate in soils than antibiotics or anticonvulsants, making them more of a concern in groundwater and surface water. Table 3 provides an overview of typical environmental fates of pharmaceuticals.

**Table 3.** Physicochemical characteristics of selected pharmaceuticals and their environmental fates and likely accumulation in plant tissues. Note that log  $K_{oc}$  is not given due to its variability between soil types. Data from Carvalho et al. 2014, Drillia et al. 2005, Jones et al. 2006, Kang et al. 2013, Tanoue et al. 2012, Thiele-Bruhn 2003, Walters et al. 2010, and X. Wu et al. 2012.

Pharmaceutical	MW (g/mol)	pK <sub>a</sub>	log K <sub>ow</sub>	Half Life (d)	Environmental Fate	Accumulation in Plants
Erythromycin	734	8.8	3.06	30	Soil	-
Ofloxacin	361	5.97-8.28	0.35	360-1386	Soil	-
Tetracycline	444	3.3	-1.3	55-578	Soil	Leaves
Oxytetracycline	460	3.27	-0.9	44	Soil	Roots
Chlortetracycline	479	3.3	-0.53	17-46	Soil	Leaves
Sulfamethoxazole	253	1.83, 5.6	0.48	-	Ground water, surface water	Roots
Sulfamonomethoxine	280	2, 6	0.2	-	Ground water, surface water	Cotyledons, roots
Sulfadimethoxine	310	2.13, 6.08	1.17	-	Ground water, surface water	Roots
Sulfadiazine	250	6.36	-0.09	1.6	Ground water, surface water	Roots
Sulfamethazine	278	7.59	0.89	74-462	Ground water, surface water	Roots
Trimethoprim	290	3.23, 6.76	0.73	22-41	Surface water	Cotyledons, roots
Carbamazepine	236	7	2.45	75-495	Soil and water	Leaves
Diclofenac	296	4.18	4.06	20	Ground water, surface water	No significant accumulation
Indomethacin	357	4.5	4.23	-	Ground water, surface water	No significant accumulation
Ibuprofen	206	4.31	3.72	6	Ground water, surface water	No significant accumulation
Ketoprofen	254	4.45	3	-	Ground water, surface water	No significant accumulation
Naproxen	230	4.15	3.18	17	Ground water, surface water	Leaves

### **Pharmaceutical uptake into plant tissue**

Pharmaceutical compounds have been detected in the roots, stems, leaves, and seeds of plants grown in soil treated with biosolids (Prosser et al. 2014). In a recent study, 8 out of 141 pharmaceuticals and personal care products studied were detected in plant tissue from vegetables grown in agricultural fields (Sabourin et al. 2012). The degree of plant uptake varies among pharmaceutical compounds.

Membrane permeability plays a large role in the ability of a plant to take up a compound and store it in its tissue. More lipophilic compounds have a greater ability to pass through phospholipid membranes, but studies have shown that compounds that are somewhere between lipophilic and hydrophilic have a greater ability to partition into plant tissues (Tanoue et al. 2012). Soil conditions also have an effect on the ability of a plant to take up pharmaceutical compounds. For example, a soil's pH can affect the ionization state of compounds it contains, which can lead to those compounds becoming more or less able to cross biological membranes. Tanoue et al. (2012) found that pharmaceuticals are readily taken up when they are near their neutral ionization states. Additionally, higher organic matter can lead to compounds binding more tightly to soil and resisting uptake (Table 3).

Antibiotics, particularly sulfonamides and trimethoprim, have been shown to be readily taken up into plants, where they are found in highest concentrations in the roots (see Figure 3). This has been demonstrated in recent laboratory and greenhouse studies on cucumber, millet, pea, corn, and cabbage (Tanoue et al. 2012). However, studies conducted in the field generally find lower concentrations of antibiotics in plant tissue. For example, the concentrations of 5 antibiotics in 11 vegetable crops treated with pharmaceutical-containing manure were found to be less than 10 µg/kg. This could be due to pharmaceuticals binding to organic matter in soils as well as a greater availability of uncontaminated subsurface area into which roots can extend (Kang et al. 2013).

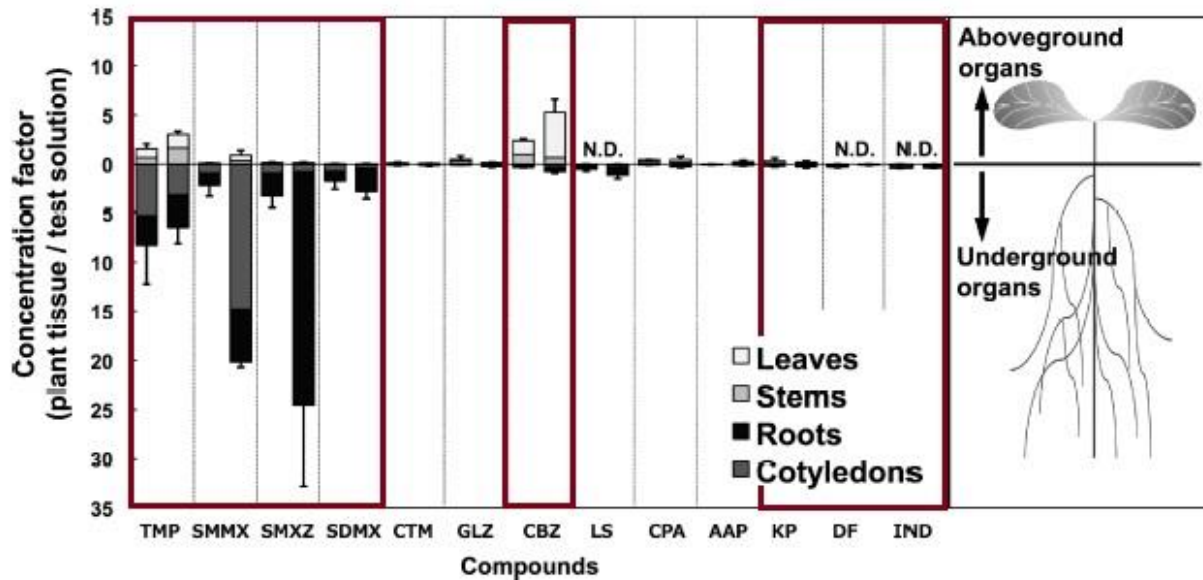
The anticonvulsant carbamazepine was described as having an "optimal" pH-independent  $K_{ow}$  for partitioning into plant stems and leaves, where it was found in relatively large quantities (Tanoue et

al. 2012). This was also predicted by two plant uptake models in a study by Prosser et al. (2014). The Dynamic Plant Uptake (DPU) Model consisted of a water and soil transport model coupled with a cell model to calculate partition coefficients and estimate transport of pharmaceuticals to plant tissues. The Biosolids-Amended Soil Level IV (BASL4) Model was a fugacity model that assessed the environmental fate of chemicals from land-applied sludge in soils and the relative concentrations of those chemicals in receptor tissue. Both the DPU and BASL4 models used standard parameters such as precipitation, temperature, soil properties, and plant tissue characteristics, and both models predicted that carbamazepine would be found in plant tissues, particularly leaves (Prosser et al. 2014). These predictions were confirmed by other studies (Carvalho et al. 2014, C. Wu et al. 2012). For example, carbamazepine was detected in stems and leaves at concentrations up to about 1,300 µg/kg in peppers, 800 µg/kg in collards, and 600 µg/kg in lettuce (C. Wu et al. 2012), confirming model predictions. As previously mentioned, carbamazepine is a ubiquitous compound in wastewater and sewage sludge and is frequently detected in soils rather than in the aqueous phase. This common distribution, as well as the pharmaceutical's physicochemical components, leads to a relatively large uptake potential for the anticonvulsant.

Compared to antibiotics and the anticonvulsant carbamazepine, NSAIDs have a relatively low detection rate in plant tissues. In one study, neither ibuprofen nor diclofenac were detected in the leaves of lettuce or spinach, while naproxen was only detected in the leaves of spinach at the relatively low concentration of 0.04 µg/kg. For comparison, in the same study carbamazepine was detected at 28.7 µg/kg in lettuce and 2.9 µg/kg in spinach leaves (X. Wu et al. 2012). Ibuprofen and naproxen have been shown to be taken up into alfalfa tissue, but only in small amounts (Carvalho 2014). One study detected neither diclofenac nor indomethacin in the stems or leaves of pea plants, and those compounds were found only at very low levels in roots. Ketoprofen was found in roots and shoots at comparatively low levels (Tanoue et al. 2012). The low detection of NSAIDs could be attributable to their

lower concentration in soils, biodegradation, and ionization states. See Figure 3 for a comparison of the concentration of pharmaceutical groups in plant tissue.

**Fig. 3.** Pharmaceutical concentrations in roots and shoots. Trimethoprim (TMP), sulfamonomethoxine (SMMX), sulfamethoxazole (SMXZ), and sulfadimethoxine (SDMX) are antibiotics; carbamazepine (CBZ) is an anticonvulsant; and ketoprofen (KP), diclofenac (DF), and indomethacin (IND) are NSAIDs. See Table 3 for the physicochemical properties of these compounds. Adapted from Tanoue et al. 2012.



### Potential effects of pharmaceuticals in land applied sludge

Pharmaceuticals in land applied sludge can affect water quality, soil microbial activity, and plant growth. Potential human and ecological health effects may exist, but these are not fully understood. As previously discussed, pharmaceuticals contained in land applied sludge can run off in stormwater to contaminate surface waters, leach into groundwater to contaminate aquifers, accumulate in soils, and/or be taken up into plant tissues.

In surface waters, many pharmaceuticals may undergo hydrolysis, photodegradation, or biodegradation, but constant re-introduction of these compounds can lead to significant exposure of aquatic organisms (Daughton 2003). Aquatic organisms are exposed to pharmaceutical compounds over their life cycles, meaning that they could be susceptible to both acute and chronic toxic effects. Studies have shown that pharmaceutical compounds can be acutely toxic to algae, microorganisms, and fish. However, because pharmaceuticals are generally detected in surface waters at the range of  $10^3$ - $10^7$

times lower than known LC<sub>50</sub> and EC<sub>50</sub> values, acute toxicity of pharmaceuticals in surface waters is not a major concern at this time (Fent et al. 2006).

Less is known about the chronic effects of repeated exposure to low levels of pharmaceutical compounds in surface waters (Daughton 2003). When comparing the concentrations of pharmaceuticals typically found in surface waters with the available chronic toxicity data for *Daphnia* and other standard laboratory tests, environmental levels do not appear to pose a chronic toxicity threat (Fent et al. 2006). Some studies have shown that certain pharmaceutical compounds may interfere with hormonal pathways in aquatic organisms (Topp et al. 2008). Additionally, bioaccumulation of diclofenac in animal tissue has been shown in some studies, which indicates that it has the potential to travel through trophic levels (Fent et al. 2006). If such is the case, bioaccumulation could lead to exposure of more organisms than those found only in aquatic environments.

Groundwater contamination with pharmaceutical compounds could be of concern to human health for those who retrieve drinking water from groundwater wells. Although water filtration plants have shown relatively effective removal of some pharmaceuticals, many compounds still pass through treatment, and those who live in rural areas may get drinking water from wells without treatment (Fent et al. 2006). This could lead to human exposure to trace amounts of pharmaceutical compounds at levels which are not thought to be acutely toxic, but more research is needed on the potential for chronic toxicity and repeat exposure (Daughton 2003).

Antibiotics in soils have gained the attention of the scientific community because of their interference with the activity of naturally-occurring soil microbes. Pala-Ozkok et al. showed that erythromycin obstructed substrate binding sites for microbes, inhibiting their activity (2013). The toxic dose of antibiotics for microorganisms is far lower than that for higher organisms, meaning that the potential for pharmaceutical toxicity is higher (Thiele-Bruhn 2003). This is significant because soil microbes are often responsible for breaking down xenobiotics, such as other pharmaceutical

compounds. Without this microbial activity, pharmaceuticals may accumulate in greater amounts. Furthermore, fluoroquinolones have been shown to bioaccumulate in soil microbes (Thiele-Bruhn 2003). Another major concern regarding antibiotics in soil matrices is the potential for antibiotic resistance. Many antibiotics, especially ciprofloxacin, have been shown to increase antibiotic resistance of soil bacteria. This antibiotic resistance proved to be short-lived and only lasted until the source was removed, but areas where antibiotics are continually re-introduced through the application of biosolids are a potential concern (Clarke and Smith 2011). Bacterial antibiotic resistance is a concern for human health because it can decrease the effectiveness of antibiotics as a treatment for infections (Carvalho et al. 2014).

Interference with soil microbes may also lead to problems with plant growth. It has been postulated that reduced soil microbe activity may lead to slower decomposition, nitrification, and nutrient cycling. This impairment of soil function could interfere with plant growth (Carvalho et al. 2014). Pharmaceuticals, especially antibiotics, have shown some phytotoxicity through biomass reduction (Carvalho et al. 2014, Eggen et al. 2011). However, it must be noted that the phytotoxicity and reductions in plant growth seen in some studies may not represent the probable effects of pharmaceuticals in land applied sludge in actual agricultural fields. Some field studies have indicated that the negative effects seen in laboratory studies were not as pronounced *in vivo* because laboratory studies use large concentrations of pharmaceuticals unlikely to be found in actual biosolids (Sabourin et al. 2012, Thiele-Bruhn 2003).

Although it has been demonstrated that pharmaceutical compounds can be taken up into crop plants and vegetables, current research shows that the likelihood of concentrations of pharmaceuticals in edible plants posing a significant human health risk is minimal (Carvalho et al. 2014, Clarke and Smith 2011, Sabourin et al. 2012, Thiele-Bruhn 2003). Antibiotic resistance in microbes found in surface water, groundwater, and soils is of more concern because of the potential for reduced effectiveness of human

drugs against dangerous pathogens (Thiele-Bruhn 2003). Because pharmaceutical compounds' potential for chronic toxicity at low, repeated doses is unknown, chronic human health effects cannot be fully assessed at this time (Daughton 2003). Even though human health risks are not fully understood, methods of removing pharmaceuticals from the waste stream have been investigated as a precautionary measure.

### **Treatment and removal of pharmaceuticals: New methods**

In recent years, a great number of studies have been conducted on the removal of emerging compounds, like pharmaceuticals, from effluent at WWTPs. For example, graphene adsorption reactor technology removed carbamazepine, diclofenac, and ibuprofen by 96% compared to the 62% removal rate of ultraviolet filtration (Rizzo et al. 2015). Membrane filtration enhanced with carbon nanotubes produced effluent with less than 10% of pharmaceutical compounds detected (Gethard et al. 2012). These new technologies can also reduce toxicity of effluent by a significant degree (Rizzo et al. 2015). Fewer studies have focused on removing pharmaceutical compounds from sludge destined for land application.

An interesting new technology focusing on fungi as an alternative to physical or chemical treatment has shown promise. White rot fungi, or *Trametes versicolor*, break down lignin in wood through an enzymatic process involving laccases and peroxidases. *T. versicolor* has a cytochrome P450 system similar to that of mammals that allows it to break down ingested compounds as well (Rodríguez-Rodríguez et al. 2011). These properties have led to the study of *T. versicolor* as a method of biodegradation and toxicity reduction of pharmaceutical compounds in sewage sludge.

In a recent study, pharmaceutical-spiked sterile sewage sludge was inoculated with *T. versicolor* in a solid-phase bioreactor to evaluate potential for degradation and toxicity reduction. Out of 14 pharmaceuticals tested, 7 were completely removed, including the antibiotics clarithromycin and sulfamethoxazole. The anticonvulsants diazepam and carbamazepine were both removed by 43%, while

the NSAIDs ibuprofen, diclofenac, and mefenamic acid were removed by 75%, 64%, and 72%, respectively (Rodríguez-Rodríguez et al. 2011). This method proved to be far more effective for antibiotics than previous studies on anaerobic digestion and natural attenuation, where sulfamethazine was removed by 0-43% (Rodríguez-Rodríguez et al. 2011). Toxicity was also reduced by 56% in standard *Daphnia magna* tests, and completely eliminated in *Vibrio fischeri* tests (Rodríguez-Rodríguez et al. 2011).

Rodríguez-Rodríguez et al. (2012) conducted a second study with *T. versicolor* focusing on non-sterile sewage sludge in which removal rates of up to 85% for clarithromycin, up to 9% for carbamazepine, up to 60% for diclofenac, and up to 61% for ibuprofen were observed. While *T. versicolor* survived well in the sewage sludge, it is likely that the naturally-occurring microbiota may have interfered with its ability to remove pharmaceutical compounds from sludge (Rodríguez-Rodríguez et al. 2012). This suggests that a preliminary sanitization step might be useful in the implementation of *T. versicolor* biodegradation treatment. Research into treatment with fungi is ongoing and may lead to more effective removal of a greater amount of compounds with further refinement.

## Conclusions

Pharmaceutical compounds are a pollutant of concern which merit scientific research and regulatory review due to their ubiquitous nature, ability to bypass treatment, and potential for human and ecological health impacts. Currently, although research into these compounds has accelerated in recent years, the behavior and effects of pharmaceuticals in the environment is not well understood. Without fail, all of the research papers reviewed in this study recommended further investigation into the environmental fate and impacts of pharmaceutical compounds.

When pharmaceuticals pass through WWTPs and are land applied in sludge, a large number of diverse and poorly understood factors affect their ability to reach human and ecological populations, and these factors should be studied further. The potential for health effects of pharmaceuticals at repeated, low doses should also be investigated, including long-term multigenerational effects. Antibiotic resistance should be of particular concern, as it is a phenomenon that can occur in any of the environmental matrices discussed in this paper and may have significant effects on our ability to fight disease (Thiele-Bruhn 2003).

As the use of pharmaceutical compounds increases, research into their removal from the waste stream, effluent, and sludge should be emphasized. New technologies have shown promise, but may be cost-prohibitive on a large scale (Rizzo et al. 2015). In addition to WWTP upgrades and sludge treatment, methods of public outreach may have beneficial results. Preventing pharmaceuticals from entering the waste stream via improper disposal could prove helpful. For example, in 2013, 324 tons of drugs were turned in during the “National Prescription Take-Back Day” sponsored by the Drug Enforcement Agency (DEA) (Wick 2014). Public education may not remove improper disposal as a source of pharmaceuticals, but it is at least a start. Further research into all areas of pharmaceutical pass through will increase our understanding, regulation, and treatment of these emerging pollutants of concern, which is a necessary step in the right direction.

## References

- Carvalho PN, Basto MCP, Almeida CMR, Brix H. 2014. A review of plant-pharmaceutical interactions: from uptake and effects in crop plants to phytoremediation in constructed wetlands. *Environ Sci Pollut Res* 21: 11729-11763. DOI 10.1007/s11356-014-2550-3.
- Chefetz B, Mualem T, Ben-Ari J. 2008. Sorption and mobility of pharmaceutical compounds in soil irrigated with reclaimed wastewater. *Chemosphere* 73: 1335-1343. DOI: 10.1016/j.chemosphere.2008.06.070.
- Clarke BO, Smith SR. 2011. Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environ Int* 37: 226-247. DOI: 10.1016/j.envint.2010.06.004.
- Daughton CG. 2003. Cradle-to-cradle stewardship of drugs for minimizing their environmental disposition while promoting human health. I. Rationale for and avenues toward a green pharmacy. *Environ Health Perspect* 111(5): 757-774. PMID: PMC1241487.
- Díaz-Cruz MS, García-Galán MJ, Guerra P, Jelić A, Postigo C, Eljarrat E, Farré M, López de Alda MJ, Petrović M, Barceló D. 2009. Analysis of selected emerging contaminants in sewage sludge. *TRAC - Trend Anal Chem* 28(11): 1263-1275. DOI: 10.1016/j.trac.2009.09.003.
- Drillia P, Stamatelatos K, Lyberatos G. 2005. Fate and mobility of pharmaceuticals in solid matrices. *Chemosphere* 60: 1034-1044. DOI: 10.1016/j.chemosphere.2005.01.032.
- Eggen T, Asp TN, Grave K, Hormazabal V. 2011. Uptake and translocation of metformin, ciprofloxacin and narasin in forage and crop plants. *Chemosphere* 85: 26-33. DOI: 10.1016/j.chemosphere.2011.06.041
- Fent K, Weston AA, Caminada D. 2006. Ecotoxicology of human pharmaceuticals. *Aquat Toxicol* 76(2): 122-159. DOI: 10.1016/j.aquatox.2005.09.009.
- Gethard K, Sae-Khow O, Mitra, S. 2012. Carbon nanotube enhanced membrane distillation for simultaneous generation of pure water and concentrating pharmaceutical waste. *Sep Pur Technol* 90: 239-245. DOI: 10.1016/j.seppur.2012.02.042.
- Gielen GJHP, van den Heuvel MR, Clinton PW, Greenfield LG. 2009. Factors impacting on pharmaceutical leaching following sewage application to land. *Chemosphere* 74: 537-542. DOI: 10.1016/j.chemosphere.2008.09.048.
- Gros M, Petrović M, Ginebreda A, Barceló D. 2010. Removal of pharmaceuticals during wastewater treatment and environmental assessment using hazard indexes. *Environ Int* 36: 15-26. DOI: 10.1016/j.envint.2009.09.002.
- Henze M, Harremoës P, Arvin E, Jansen JC. 2002. In: *Wastewater Treatment: Biological and Chemical Processes* (Förestner U, Murphy RJ, Rulkens JH, eds.). New York: Springer, 430.
- Hörsing M, Ledin A, Grabic R, Fick J, Tykslind M, Jansen JC, Andersen HR. 2011. Determination of sorption of seventy-five pharmaceuticals in sewage sludge. *Water Res* 45: 4470-4482. DOI: 10.1016/j.watres.2011.05.033.
- IMS (IMS Health Institute for Healthcare Informatics). 2012. The Use of Medicines in the United States: Review of 2011. Available: [https://www.imshealth.com/ims/Global/Content/Insights/IMS%20Institute%20for%20Healthcare%20Informatics/IHII\\_Medicines\\_in\\_US\\_Report\\_2011.pdf](https://www.imshealth.com/ims/Global/Content/Insights/IMS%20Institute%20for%20Healthcare%20Informatics/IHII_Medicines_in_US_Report_2011.pdf) [accessed 30 December 2014].
- Jasim SY, Irabelli A, Yang P, Ahmed S, Schweitzer L. 2006. Presence of pharmaceuticals and pesticides in Detroit River water and the effect of ozone on removal. *Ozone Sci Eng* 28: 415-423. DOI: 10.1080/01919510600985945.
- Jelić A, Gros M, Ginebreda A, Cespedes-Sánchez R, Ventura F, Petrović M, Barceló D. 2011. Occurrence, partition, and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. *Water Res* 45: 1165-1176. DOI: 10.1016/j.watres.2010.11.010.
- Jones OAH, Voulvoulis N, Lester JN. 2006. Partitioning behavior of five pharmaceutical compounds to activated sludge and river sediment. *Arch Environ Contam Toxicol* 50: 297-305. DOI: 10.1007/s00244-005-1095-3.
- Jones V, Gardner M, Ellor B. 2014. Concentrations of trace substances in sewage sludge from 28 wastewater treatment works in the UK. *Chemosphere* 111: 478-484. DOI: 10.1016/j.chemosphere.2014.04.025.

- Jones-Lepp TL, Stevens R. 2007. Pharmaceuticals and personal care products in biosolids/sewage sludge: the interface between analytical chemistry and regulation. *Anal Bioanal Chem* 387: 1173-1183. DOI: 10.1007/s00216-006-0942-z.
- Kang DH, Gupta S, Rosen C, Fritz V, Singh A, Chander Y, Murray H, Rohwer C. 2013. Antibiotic uptake by vegetable crops from manure-applied soils. *J Agric Food Chem* 61: 9992-10001. DOI: 10.1021/jf404045m.
- Kinney CA, Furlong ET, Zaugg SD, Burkhardt MR, Werner SL, Cahill JD, Jorgensen GR. 2006. Survey of organic wastewater contaminants in biosolids destined for land application. *Environ Sci Technol* 40: 7207-7215. DOI: 10.1021/es0603406.
- Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. *Environ Sci Technol* 36: 1202-1211. DOI: 10.1021/es011055j.
- Martín J, Santos JL, Aparicio I, Alonso E. 2015. Pharmaceutically active compounds in sludge stabilization treatments: Anaerobic and aerobic digestion, wastewater stabilization ponds, and composting. *Sci Total Environ* 503-504: 97-104. DOI: 10.1016/j.scitotenv.2014.05.089.
- Nieto A, Borrull F, Pocurull E, Marcé RM. 2007. Pressurized liquid extraction of pharmaceuticals from sewage-sludge. *J Sep Sci* 30: 979-984. DOI: 10.1002/jssc.200600360.
- Nieto A, Borrull F, Pocurull E, Marcé RM. 2010. Pressurized liquid extraction: A useful technique to extract pharmaceuticals and personal-care products from sewage sludge. *TRAC - Trend Anal Chem* 29(7): 752-764. DOI: 10.1016/j.trac.2010.03.014.
- Pala-Ozkok I, Ubay-Cokgor E, Cakar ZP, Orhon D. 2014. Acute impact of erythromycin on substrate utilization by activated sludge: effect of sludge age. *J Chem Technol Biotechnol* 89: 1091-1102. DOI: 10.1002/jctb.4208.
- Prosser RS, Trapp S, Sibley PK. 2014. Modeling uptake of selected pharmaceuticals and personal care products into food crops from biosolids-amended soil. *Environ Sci Technol* 48: 11397-11404. DOI: 10.1021/es503067v.
- Rizzo L, Fiorentino A, Grassi, M, Attanasio D, Guida, M. 2015. Advanced treatment of urban wastewater by sand filtration and graphene adsorption for wastewater reuse: Effect on a mixture of pharmaceuticals and toxicity. *J Environ Chem Eng* 3: 122-128. DOI: 10.1016/j.jece.2014.11.011.
- Rodríguez-Rodríguez CE, Jelić A, Llorca M, Farré M, Caminal G, Petrović M, Barceló D, Vicent T. 2011. Solid-phase treatment with the fungus *Trametes versicolor* substantially reduces pharmaceutical concentrations and toxicity from sewage sludge. *Bioresource Technol* 102: 5602-5608. DOI: 10.1016/j.biortech.2011.02.029.
- Rodríguez-Rodríguez CE, Jelić A, Pereira MA, Sousa DZ, Petrović M, Alves MM, Barceló D, Caminal G, Vicent T. 2012. Bioaugmentation of sewage sludge with *Trametes versicolor* in solid-phase biopiles produces degradation of pharmaceuticals and affects microbial communities. *Environ Sci Technol* 46: 12012-12020. DOI: 10.1021/es301788n.
- Sabourin L, Duenk P, Bonte-Gelok S, Payne M, Lapen DR, Topp E. 2012. Uptake of pharmaceuticals, hormones, and parabens into vegetables grown in soil fertilized with municipal biosolids. *Sci Total Environ* 431: 233-236. DOI: 10.1016/j.scitotenv.2012.05.017.
- Tanoue R, Sato Y, Motoyama M, Nakagawa S, Shinohara R, Nomiyama K. Plant uptake of pharmaceutical chemicals detected in recycled organic manure and reclaimed wastewater. *J Agr Food Chem* 60: 10203-10211. DOI: 10.1021/jf303142t.
- Ternes TA. 1998. Occurrence of drugs in German sewage treatment plants and rivers. *Water Res* 32(11): 3245-3260. PII: S0043-1354(98)00099-2.
- Thiele-Bruhn S. 2003. Pharmaceutical antibiotic compounds in soils – a review. *J Plant Nutr Soil Sci* 166: 145-167. DOI: 10.1007/s00373-003-0204-145.
- Topp E, Monteiro SC, Beck A, Coelho BB, Boxall ABA, Duenk PW, Kleywegt S, Lapen DR, Payne M, Sabourin L, Li H, Metcalfe CD. 2008. Runoff of pharmaceuticals and personal care products following application of biosolids to an agricultural field. *Sci Total Environ* 396: 52-59. DOI: 10.1016/j.scitotenv.2008.02.011.
- UNEP DTIE (United Nations Environment Programme Division of Technology, Industry and Economics). 2002. *Biosolids Management: An Environmentally Sound Approach for Managing Sewage Treatment Plant Sludge*,

- An Introductory Guide to Decision-Makers. 1st ed. Osaka: UNEP DTIE International Environmental Technology Centre. Available: <http://www.unep.or.jp/ietc/publications/freshwater/fms1>.
- USEPA (US Environmental Protection Agency). 1996. Soil Screening Guidance: Technical Background Document. Part 5: Chemical-Specific Parameters. Available: [http://www.epa.gov/superfund/health/conmedia/soil/pdfs/part\\_5.pdf](http://www.epa.gov/superfund/health/conmedia/soil/pdfs/part_5.pdf) [accessed 5 March 2015].
- USEPA (US Environmental Protection Agency). 2007. Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS. Available: [http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2008\\_01\\_03\\_methods\\_method\\_1694.pdf](http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2008_01_03_methods_method_1694.pdf) [accessed 17 January 2015].
- USEPA (US Environmental Protection Agency). 2014. Toxic and Priority Pollutants. Available: <http://water.epa.gov/scitech/methods/cwa/pollutants-background.cfm> [accessed 30 November 2014]
- Uslu MO, Biswas N, Jasim S. 2011. Chemicals of emerging concern in the Great Lakes region. International Joint Commission: 72 pp.
- Walters E, McClellan K, Halden RU. 2010. Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. *Water Res* 44: 6011-6020. DOI: 10.1016/j.watres.2010.07.051.
- Wick C. 2014. Mandatory drug take-back programs: Will they survive the dormant commerce clause challenge? *Tulane Env Law J* 27: 371-391.
- Wu C, Spongberg AL, Witter JD, Sridhar BBM. 2012. Transfer of wastewater associated pharmaceuticals and personal care products to crop plants from biosolids treated soil. *Ecotox Environ Safe* 85: 104-109. DOI: 10.1016/j.ecoenv.2012.08.007.
- Wu X, Conkle JL, Gan, J. 2012. Multi-residue determination of pharmaceutical and personal care products in vegetables. *J Chromatogr A* 1254: 78-86. DOI: 10.1016/j.chroma.2012.07.041.
- Xu J, Wu L, Chang AC. 2009. Degradation and adsorption of selected pharmaceutical and personal care products (PPCPs) in agricultural soils. *Chemosphere* 77: 1299-1305. DOI: 10.1016/j.chemosphere.2009.09.063.
- Yu Y, Wu L. 2012. Analysis of endocrine disrupting compounds, pharmaceuticals, and personal care products in sewage sludge by gas chromatography-mass spectrometry. *Talanta* 89: 258-263. DOI: 10.1016/j.talanta.2011.12.023.