UPTAKE AND ACCUMULATION OF PHARMACEUTICALS IN SURFACE AND OVERHEAD IRRIGATED LETTUCE

By

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ABSTRACT

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Crop irrigation with reclaimed water is becoming increasingly popular due to water shortages exacerbated by climate change and variability. Pharmaceuticals in reclaimed water may pose unintended food safety risks when they accumulate in crops. Understanding the uptake pathways and accumulation levels of pharmaceuticals in crops under typical irrigation practices is critical for accurate risk assessment of crop irrigation using reclaimed water. The objectives of this study were to investigate the uptake of pharmaceuticals by greenhouse-grown lettuce irrigated with pharmaceutical-contaminated water via overhead or surface irrigation. Eleven commonly used pharmaceuticals, including a fever reducer and pain reliever (acetaminophen), a stimulant (caffeine), an anticonvulsant (carbamazepine), and 8 antibiotics (sulfadiazine, sulfamethoxazole, carbadox, trimethoprim, lincomycin, oxytetracycline, monensin sodium, and tylosin) were selected. Lettuce plants were grown for 5 weeks in nursery pots in a greenhouse, and pharmaceutical concentrations in roots, shoots, soil, and irrigation water were analyzed to infer major uptake pathways. Results showed that pharmaceuticals with low lipophilicity, low molecular weight, and high water solubility (acetaminophen, caffeine, carbamazepine, sulfadiazine, sulfamethoxazole, and carbadox) had no significant difference in shoot concentration overtime between irrigation methods. Conversely, those pharmaceuticals with high lipophilicity, high molecular weight, and lower water solubility (monensin sodium and tylosin) showed a higher concentration in shoots of overhead-irrigated compared to surface-irrigated plants.

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KEY TO ABBREVIATIONS

CECs: chemicals of emerging concern

WWTPs: wastewater treatment plants

CAFOs: concentrated animal feeding operations

IPM: integrated pest management

FDA: Food and Drug Administration

DI: deionized

MeOH: methanol

Na_2SO_4: anhydrous sodium sulfate

PSA: primary-secondary amine

Na_2EDTA: disodium ethylenediaminetetraacetate

NaCl: sodium chloride

HLB: hydrophilic-lipophilic balance

QuEChERS: quick, easy, cheap, effective, rugged, and safe

CHAPTER ONE: LITERATURE REVIEW

INTRODUCTION

Pharmaceuticals have been recognized as emerging contaminants or chemicals of emerging concern (CECs) (EPA, 2015), because of their widespread use, constant discharge into the environment, and possible risks to human and ecosystem health. Pharmaceuticals include antibiotics and other human and animal medicines for disease treatment or prevention. Antibiotics are an important class of pharmaceuticals, and are used in the treatment and prevention of bacterial infections by either killing bacteria or inhibiting their growth (Sarmah et al., 2006). They can occur naturally in the environment by soil microorganisms and fungi, or be introduced by anthropogenic activities. They are being released into the environment via two major pathways, human and animal waste. Since animals do not metabolize antibiotics fully, the remainder can end up on agricultural land where manure has been applied as fertilizer (Hirsch et al., 1999). They can also be introduced to the environment via land application of biosolids or effluents from wastewater treatment plants (WWTPs) (Yi et al., 2011; Estévez et al., 2012; Clarke and Porter, 2010).

Antibiotics have been found in various water sources such as wastewater effluents, surface waters, and groundwater (Hirsch et al., 1999; Tanoue et al., 2012). Wastewater effluents are sometimes used for crop irrigation, especially in water-stressed regions (Khan et al., 2008; Toze, 2006; Yi et al., 2011). This practice is becoming increasingly popular in many parts of the world due to water shortages and long-duration droughts, exacerbated by climate change and variability (Pedrero et al., 2010; Pereira et al. 2002; Yi et al., 2011), and can be a major pathway for introduction of pharmaceuticals into agricultural land and food crops. The release of pharmaceuticals to agricultural environments increases the risk of antibiotic resistance in microbes and potential human exposure through uptake into crops destined for human consumption.

SOURCES OF PHARMACEUTICALS IN THE ENVIRONMENT

Livestock. Antibiotics play an important role in modern agriculture, specifically in concentrated animal feeding operations (CAFOs) where low doses of antibiotics are given to livestock to prevent disease and promote growth. In 2015, 89.8 million cattle were raised for meat and milk production, generating an estimated $44 billion in economic impact (Beef USA, 2016). In the same year, 66.9 million swine (USDA, 2015a) and over 8 million poultry (USDA, 2015b) were produced. High production volume and close animal quarters can promote the spread of disease, resulting in the pervasive use of antibiotics in livestock production.

Antibiotics and other pharmaceuticals are commonly administered to animals via subcutaneous injection, oral injection, feed or drinking water (Sarmah et al., 2006). The most common pharmaceuticals, such as tetracyclines, sulfonamides, and ionophores (FDA, 2015), are used to treat or prevent diseases and infections, promote growth, and manage reproduction. Unfortunately, their widespread use has led to their transfer from agricultural areas into the environment through runoff or land application of manure.

In a 2013 Food and Drug Administration (FDA) Summary Report, antimicrobials were divided into two categories as they pertain to humans: medically important and non-medically important. The domestic sale and distribution of non-medically important antimicrobials for livestock are reported to increase by 14% from 2009 through 2013 in domestic sales (FDA, 2015). During this time, the sale and distribution of medically important antimicrobials also increased by 20%, from about 7.6 to 9.1 million kg. Medically important antibiotics such as tetracyclines and penicillins accounted for 71% and 9% of sales to the livestock industry, respectively. While sale and distribution data are helpful for making predictions about antibiotic use, actual use is unknown. For example, veterinarians are able to prescribe drugs for uses other than those specified on the label.

Some antibiotics are poorly absorbed by animals. As much as 30-90% can be excreted in feces or urine (Sarmah et al., 2006). Metabolites can also be excreted and can potentially still be bioactive. Kim et al. (2011) found that excretion rates in cattle varied with antibiotic type, with 75% excretion for sulfamethazine and 50-100% for tylosin. Excretion rates also depend on species and route of application (Kemper, 2008). For example, sheep can excrete up to 20% of oxytetracycline when administered orally and cattle can excrete 17-75% of chlortetracycline (Montforts et al., 1999; Jjemba, 2002). These differences can result in varying concentrations of antibiotics in waste material and consequently in the environment from livestock production.

Pharmaceuticals are usually highly concentrated in manure and less likely than biosolids to be degraded quickly since there is virtually no processing (Carvalho et al., 2014). Some pharmaceuticals, such as monensin sodium and tylosin, have been shown to degrade during storage, but others, such as sulfadiazine and difloxacin in pig manure, have shown no decrease in concentration after 150 days of storage (Carvalho et al., 2014). Since pharmaceutical degradation in biosolids and manure can be slow, application onto arable land results in pharmaceutical loading.

Human. Widespread human use of antibiotics to treat bacterial infections has led to their introduction into the environment via municipal WWTPs. Through excretion in waste and improper disposal of unused medications, bioactive antibiotics can enter wastewater influents (Ternes, 1998).

Through screening, agitation, aeration, and chlorination technologies, WWTPs effectively remove over 90% of suspended solids and 99% of harmful bacteria (EPA, 1998; USGS, 2015). Unfortunately, the standard treatment process is not designed to filter out or deactivate pharmaceuticals, resulting in contaminated effluents. These effluents are also often referred to as reclaimed water if intended for beneficial use. Contaminated effluents are then released into surface waters or used for irrigation directly (Yi et al., 2011; Estévez et al., 2012).

In a 2010 survey of a Wastewater Treatment Facility at East Lansing, MI, Gao et al. (2012) analyzed the influents and effluents for the presence of various antibiotics. They found sulfamethoxazole, a sulfonamide antibiotic, at a concentration of 1566 ng/L in the raw influent and 178 ng/L in the final effluent. They also found that the average concentration of lincomycin, a lincosamide antibiotic, was 58 ng/L in the raw influent and 35 ng/L in the final effluent. In another study, Gros et al. (2010) analyzed effluents from seven WWTPs along the Ebro river in Northeastern Spain; they detected pharmaceuticals in 100% of their samples. Both studies highlight that pharmaceuticals are being emitted into water sources from WWTPs and that conventional methods of wastewater treatment are unable to remove all pharmaceuticals effectively.

Another example is provided by Yi et al. (2011), who describe how, driven by increasing population and water scarcity, reclaimed waste water use increased in many regions of China. China began using reclaimed water in the 1940s and since then has used it for everything from irrigation to industrial processes. As water scarcity becomes a global problem, reclaimed water may become a widely used source of water. These examples highlight that pharmaceuticals are increasingly contaminating water sources and reaching environments that are closely linked to food production.

Biosolids, or sewage sludge, is the solid portion of treated sewage from WWTPs (EPA, 2016). Biosolids are rich in nutrients and organic matter and are often contaminated with pharmaceuticals. It is common practice to recycle them and add them to agricultural lands as a fertilizer, which also unintentionally results in pharmaceutical loadings. Manure is used in a similar manner and can also be contaminated; both are used to improve or rebuild poor soils.

Aquaculture. Aquaculture, or fish farming, includes the breeding, rearing, and harvesting of plants and animals in all types of aquatic environments (NOAA, 2012). Aquaculture is practiced globally in both fresh and salt water systems. This type of farming produces vast amounts of commercial edible fish and eggs, bait fish, and ornamental fish. In the U.S., marine aquaculture primarily produces oysters, clams, mussels, shrimp, and salmon. On the other hand, freshwater aquaculture produces catfish, trout, tilapia, and bass. Both industries may overlap with natural environments, such as utilizing cages on the ocean floor or natural ponds, or take place inconstructed facilities, such as recirculating aquaculture systems.

Demand for food and depletion of natural fish supplies has led to increasing popularity of global aquaculture and rapid growth of the industry (Cabello, 2006). Aquaculture practices include the use of large amounts of pharmaceuticals to manage disease and infection. Because of the proximity of natural fish environments and human-managed aquaculture, there is a significant risk of pharmaceuticals entering water sources. For example, in samples from shrimp aquaculture ponds in Vietnam, researchers detected high levels of trimethoprim (up to 2.03 mg/L in water and 734.61 mg/kg in mud), sulfamethoxazole (up to 5.57 mg/L in water and 820.49 mg/kg in mud), norfloxacin (up to 6.06 mg/L in water and 2615.96 mg/kg in mud) and oxolinic acid (up to 2.50 mg/L in water and 426.31 mg/kg in mud) and also found antibiotic residues in canals close to the ponds (Le and Munekage, 2004).

Fish are administered antibiotics through feed, the water, or injections (Cabello, 2006). Fish waste and unconsumed food that contain antibiotics can make contact with sediment and can potentially move into surrounding aquatic environments. Residual antibiotics can be consumed by non-targeted aquatic life, remain in sediments or water, and apply selective pressure to microorganisms.

Pharmaceuticals used in aquaculture contribute to environmental contamination. Since aquaculture production in the U.S. is relatively small compared to global aquaculture, antibiotics are thought to not presently play a large role in domestic environmental contamination, but data are scarce (Kümmerer, 2009).

Antibiotic Use on Crops. Antibiotics have been useful for controlling bacterial infections and diseases on crops and ornamental plants (Misra, 1986). Bacterial diseases of plants are less common than fungal or viral infections, and therefore, antibiotics are only utilized in well-defined circumstances (Vidaver, 2002). Historically, many antibiotics were commonly used to treat a variety of plant diseases. For example, penicillin was widely used to treat crown-gall bacterium and tetracycline was used against tomato canker (Misra, 1986). As of 2002, the two most common antibiotics used in crops are streptomycin and oxytetracycline. Antibiotics are most commonly utilized against *Erwinia amylovora* which causes fire blight in pear and apple, and *Xanthomonas campestris*, which causes bacterial spot on peach (Stockwell and Duffy, 2002). Since orchards are a large economic investment for growers, the potential loss resulting from pathogen infection warrants timed antibiotic sprays.

Millions of kilograms of antibiotics are used in the U.S. annually, of which only about 0.1% are used in plant agriculture (Vidaver, 2002; Stockwell and Duffy, 2002). For example, in 2009, about 16,000 kg of antibiotics were used for orchards, which was only 0.12% of the total antibiotics used in animal agriculture. Therefore, not much attention has been paid to antibiotic use on crops with respect to soil or water contamination although usage numbers are high. In another example, in 1999, 30% of total national pear orchard acres received about 2,700 kg of streptomycin and 40% of total pear orchard acres received about 5,400 kg of oxytetracycline as reported by the USDA (Vidaver, 2002). Apples received about 52,000 kg of streptomycin on about 20% of the total apple orchard acres or about 1,300 kg of oxytetracycline on 5% of apple orchard acres.

Bacterial resistance has occurred against antibiotics on crops; streptomycin resistant bacteria have been observed on tomatoes and peppers (Mirsa, 1986). Fire blight resistant to streptomycin resulted in limiting its use on orchards by incorporating more integrated pest management (IPM) strategies and adding new antibiotics (Stockwell and Duffy, 2002). One strategy of IPM used in orchards includes pruning and removing infected branches from orchards. Through effective management strategies, streptomycin resistance can be limited. The inclusion of IPM practices coupled with the relatively low percentage of antibiotics used on crops, means that there is relatively little attention paid to crop antibiotic use from an environmental perspective. Antibiotic residues have been detected on plant surfaces, but because the ones used specifically for crops are non-persistent and deactivated quickly by sunlight, residues on crops are not a large concern. Residue studies are also limited to streptomycin, and as of the 2000s, there have been no observations of toxicity to mammals or adverse effects to human health (Vidaver, 2002). Nonetheless, the persistence and spread of antibiotic resistant bacteria from plants remain unknown.

It is unlikely that new antibiotics will be added for use in plant agriculture because of their high costs, regulations, and health concerns, as well as the increase in biocontrol and genetically modified plants (Vidaver, 2002).

ENVIRONMENTAL DETECTION AND OCCURRENCE OF PHARMACEUTICALS

Soils and Sediments. Application of manure, biosolids, and WWTP effluents has led to non-medically important and medically important antibiotics such as tetracyclines and sulphonamides, being detected in sediments and soils (Kümmerer, 2009). Sorption, leaching and degradation are three important processes that govern pharmaceutical movement in soil and water systems. These processes are driven by their chemical properties such as size and solubility (Sarmah et al., 2006). Many pharmaceuticals are persistent and have long half-lives in soils where they are bioavailable to bacteria and plants (Carvalho et al., 2014).

Boxall et al. (2006) studied not only plant uptake, but also provided semi-quantitative evidence about antibiotic dissipation in soils. They showed that amoxicillin dissipated very quickly and florfenicol, enrofloxacin, and oxytetracycline could persist in the soil environment for over 6 months following application, highlighting the different behaviors among pharmaceuticals.

Surface Waters and Groundwater. Although low levels of pharmaceuticals are typically detected in surface and ground water, their constant addition to water systems makes them semi-persistent contaminants (Carvalho et al., 2014). Concentrations of pharmaceuticals in waters at or near discharge points from pharmaceutical manufacturers or hospitals are higher than overall environmental concentrations (Hirsch et al., 1999). For example, groundwater samples down from the Grindsted Landfill Site in Denmark, previously used for household and pharmaceutical production waste, contained a variety of sulfonamide antibiotics at concentrations up to 5 mg/L (Holm et al., 1995). In another example, the ciprofloxacin concentration in effluent samples from a WWTP near Hyderabad, India that serves about 90 drug manufacturing plants was as high as 31 mg/L (Larsson et al., 2007). Discharge from WWTPs and run-off from fields are common ways for pharmaceuticals to enter surface waters. Run-off and leaching from livestock farms are the main pathways for veterinary pharmaceuticals to enter groundwater sources (Chiu and Westerhoff, 2010).

Calderón-Preciado et al. (2011) identified 40 emerging contaminants in surface waters from northeastern Spain. Concentrations were less than 5 μg/L, and chloroform, caffeine, naproxen and galaxoide were more frequently found than others. As expected, waterbodies in close proximity to WWTPs were impacted more heavily by treated wastewater effluents and had greater contaminant concentrations than those that were further removed.

Currently, pharmaceutical exposure through groundwater consumption is not considered a high risk to human health because of low concentrations and inconsistent detection, but more research is needed (Chiu and Westerhoff, 2010; Daughton, 2010). Chiu and Westerhoff (2010) examined 26 commonly used pharmaceuticals and personal care products in surface drinking water sources, a wastewater treatment plant, and groundwater in Arizona. Groundwater samples contained contaminants at 2 to 10 ng/L with only one single site detecting sulfamethoxazole and sucralose at 20 ng/L to 1 μg/L. In surface waters, caffeine, DEET and triclosan were detected at concentrations ranging from 10 to 20 ng/L, and sucralose and oxybenzne at concentrations between 20 ng/L to 1 μg/L. Numerous compounds such as testosterone, progesterone, and ethinyl were not detected.

POTENTIAL EXPOSURE AND RISKS TO HUMAN HEALTH

Crop Uptake. In the future, as climate change progresses, some water-scarce areas will look towards new sources of water for irrigation (Pereira et al., 2002). Considering these pressures, an important question to ask is: how could using irrigation water or applying biosolids and manure contaminated with pharmaceuticals affect human and environmental health? It is clear that pharmaceuticals are being introduced into the environment and are detected in water sources, but the effects on human and environmental health are unclear. Studies show that antibiotics have been found in plants grown in soils treated with manure (Kang et al., 2013). Consumption of crops that have accumulated antibiotics is a potential pathway of human exposure.

In uptake studies by Boxall et al. (2006) carrot roots and lettuce leaves took up florfenicol and trimethoprim. Interestingly, except for trimethoprim, a majority of the pharmaceuticals were associated with the outer layer of the carrot, giving evidence for the importance of food processing in the future. It has been suggested that for neutral chemicals, hydrophobicity is the most important property involved in uptake from soil to plants (Carter et al. 2014). Carter et al. (2014) grew radish and ryegrass in soil spiked with carbamazepine, diclofenac, fluoxetine, propranolol, sulfamethazine, and triclosan. Of the six chemicals, five were detected in plant tissues. Carbamazepine was taken up in the greatest amount (52 μg/g in radish and 33 μg/g in ryegrass), likely due to its persistence in soils and high concentrations in soil pore water that resulted in its transport by mass flow.

Calderón-Preciado et al. (2013) evaluated the effect of relative humidity on foliar sorption of organic contaminants. The controlled experiment simulated overhead sprinkler irrigation of lettuce under 40 and 90% relative humidity. Lettuce leaves were sprayed with a solution containing pharmaceuticals and other contaminants. Results showed that foliar sorption of compounds was similar at both relative humidities. Neutral and basic compounds predominated in the leaf compartment, while acidic compounds were partitioned between the leaf tissue and rinse water. They concluded that volatility and polarity play a large role in the final fate of the compound. This study shows that foliar sorption of contaminants through sprinkler irrigation is a viable and potentially important route of uptake into plants.

After reviewing risks of reclaimed water use for agricultural irrigation, Fatta-Kassinos et al. (2011) concluded that there are gaps in knowledge especially around risks to non-target organisms. Antibiotic availability to organisms can be reduced in the environment by biotic and abiotic factors such as degradation and adsorption (Kim et al., 2010). In the case of metals, stomatal accumulation has been described to play a role in plant uptake, which could also possibly apply to antibiotic uptake (Xiong et al., 2014). Calderón-Preciado et al. (2013) used models complemented by experimental samples, and concluded that human exposure to emerging contaminants through fruits and vegetables ranged from 1 to more than 461 ng per person per day.

After reviewing the literature concerning pharmaceuticals in the environment, it is evident that there are wide gaps in knowledge around the environmental fate and mechanisms of uptake into plants. There is a wide diversity of plant uptake research techniques and subsequently a wide variation of pharmaceutical concentrations in plants. Results are dependent on factors such as plant species, soil type, growing conditions, and pharmaceutical concentrations in water, manure, biosolids, or soil. New human risks also emerge when considering crop irrigation methods, pharmaceutical residues in reclaimed water, and predicted fresh water scarcity. While pharmaceutical concentrations are low overall, the persistence of pharmaceuticals, pervasive occurrence from non-point sources, and high concentrations at point-source areas means that more knowledge about the fate and uptake of pharmaceuticals is needed, and there is a growing concern for human and environmental health in the future.

CHAPTER TWO: UPTAKE AND ACCUMULATION OF PHARMACEUTICALS IN SURFACE AND OVERHEAD IRRIGATED LETTUCE

INTRODUCTION

Pharmaceuticals are considered chemicals of emerging concern because of their large use and frequent detection in the environment, and possible human health risks due to unintended exposure (EPA, 2015; Hirsch et al., 1999; Yi et al., 2011). Pharmaceuticals can enter the environment through many different pathways, particularly through use in animal agriculture and human medicine. Antibiotics are used for growth promotion and to fight infections, but the administered antibiotics often cannot be fully metabolized and utilized. For example, after administration of tetracycline and sulfamethoxazole in humans, 80–90% and 10–30% can be excreted and enter municipal WWTPs, and are eventually released to the environment through biosolids (i.e., sewage sludge) and wastewater effluents (Mathews and Reinhold, 2013). They can also be excreted by animals and end up in manure (Kim et al., 2011). Both biosolids and manure are often applied on agricultural land, resulting in contamination of soil and water.

Antibiotics have been widely found in wastewater effluents, surface water, and groundwater (Hirsch et al., 1999; Tanoue et al., 2012). Currently, over 70% of the world's freshwater usage is in agricultural irrigation, but due to increasing water shortages, alternative water sources must be considered. Wastewater effluents and contaminated surface water are often used as irrigation sources for crops (Pedrero et al., 2010; Yi et al., 2011), particularly in water-stressed regions. Concerns have been raised regarding human and ecosystem health risks of non-traditional water use such as crop irrigation with reclaimed wastewater effluents (Yi et al., 2011; Tanoue et al., 2012). Understanding the transfer of pharmaceuticals from contaminated soil and water to crops and their concentration levels in crops is critical to informed assessment of exposure and health risks. Several recent studies have examined the uptake and accumulation of pharmaceuticals in plants including vegetable crops (Boxall et al., 2010; Carvalho et al., 2014; Goldstein et al., 2014; Kang et al., 2013; Kong et al., 2007). However, most of these studies were root exposure experiments (Eggen et al., 2011; Eggen and Lillo, 2012; Macherius et al., 2012; Malchi et al., 2014; Sallach et al., 2015), with little work having been directed to foliar uptake of pharmaceuticals. Two studies reported that foliar uptake most likely occurs for lipophilic compounds (Calderón-Preciado et al., 2013; Lu et al., 2015). Lu et al. (2015) observed greater accumulation of relatively lipophilic bisphenol A ($\log K_{OW} = 3.40$) and nonylphenol ($\log K_{OW} = 4.48$) in lettuce and tomato through foliar rather than subsurface root exposure. Similarly, Calderón-Preciado et al. (2013) found greater retention of lipophilic contaminants in leaves in the dark even when stomata were closed. Therefore, foliar uptake of relatively lipophilic pharmaceuticals deserves further study.

In 2013, 55.3 million acres of US agricultural land was irrigated, 94% being for harvested cropland (52 million acres) (USDA, 2014). Consequently, it is important to evaluate the effect of irrigation method on pharmaceutical uptake by crops, in particular vegetables, as vegetables are often consumed with minimal processing. Assuming consistent percentages and patterns overtime, about 51% of irrigation is by overhead sprinkler systems, 42% surface irrigation, and 7% microirrigation (USGS, 2016) making foliar exposure through overhead irrigation potentially a major pathway for pharmaceutical transfer from water to crops. Elucidating the relative magnitude of pharmaceutical uptake and accumulation in vegetables via different irrigation methods is critical to developing sustainable practices for utilizing reclaimed water for ensuring food quality and safety as well as human and environmental health.

Therefore, the objective of this study was to compare the accumulation of pharmaceuticals into lettuce irrigated with pharmaceutical-contaminated water via overhead or surface irrigation. We selected eleven commonly used pharmaceuticals, including a fever reducer and pain reliever (acetaminophen), a stimulant (caffeine), an anticonvulsant (carbamazepine), and 8 antibiotics (sulfadiazine, sulfamethoxazole, carbadox, trimethoprim, lincomycin, oxytetracycline, monensin sodium, and tylosin), because of their large use by humans and animals, and varying physicochemical properties such as molecular weight, water solubility, charge behaviors (pK_a), and hydrophobicity (log K_{OW}) (Table 1). Lettuce plants were greenhouse-grown for 5 weeks in nursery pots during which time pharmaceutical concentrations in roots, shoots, soil, and irrigation water were determined to infer major uptake pathways.

MATERIALS AND METHODS

Experimental Design. This study was conducted at the Michigan State University Horticulture Teaching Greenhouses (East Lansing, MI, USA). Lettuce was seeded in sterile potting mix, watered with deionized (DI) water, fertilized, and transplanted to individual pots filled with air-dried and sifted field soil before each trial. Thirty-six lettuce plants were grown and irrigated with a constructed automatic overhead and surface irrigation system. Two trials were performed at two varying pharmaceutical concentration levels of 50 and 30 µg/L. Trial 1 was conducted from 4/20/15 to 5/25/15, and plants were irrigated with DI water spiked with 11 pharmaceuticals of approximately 50 µg/L for each pharmaceutical. Trial 2 was conducted from 9/17/15 to 10/22/15 and plants were irrigated with nutrient solution spiked with the same 11 pharmaceuticals of 30 µg/L for each pharmaceutical. The lettuce plants were harvested weekly, and then rinsed, separated into roots and shoots, and weighed. Shoot wash water was extracted and quantified for pharmaceuticals in the 50 µg/L trial, but not for the 30 µg/L trial, as minimal pharmaceutical residues were found on the lettuce shoot surface. The soil in each pot was separated into top, middle, and bottom layers of 3-cm. All samples were stored in a −20°C freezer (Northland, Greenville, MI, USA) before being dried, weighed, ground, extracted, and quantified by LC-MS/MS.

Irrigation System Design. An automatic irrigation system with overhead and surface irrigation emitters was designed and constructed by Sangho Jeon, and tested for accuracy and uniformity before the beginning of the experiments. Water amount was tested by measuring water amount at specific time intervals. The basic system design is shown in Figure 1. With this system, pump speed controlling water flow rate, and irrigation timing and duration were controlled via a control box. By connecting the control box to a laptop computer using an Arduino programming system (https://www.arduino.cc/), essential system functions could be controlled. A chipset microcontroller with a relay was used to control irrigation time. The system was powered by Supernight™ Regulated Switching Power Supply (DC 12V 20A, AC 110-240V, Portland, OR, USA).

Main components of the system include a pump, pressure gauge, autovalve, controller, two water tanks with and without pharmaceuticals, distribution lines and emitters for overhead and surface irrigation. The pharmaceutical treatment also had an extra component: an accumulator tank to help evenly regulate irrigation and water amounts. The system accommodates 36 plants: 18 plants under overhead irrigation including 15 plants for pharmaceutical treatment and 3 plants for pharmaceutical-free treatment (i.e., control); 18 plants under surface irrigation including 15 plants for pharmaceutical treatment and 3 plants for pharmaceutical-free treatment. PC Woodpecker Dripper emitters (1.0 GPH, Netafim product # 01wpc4, Tel Aviv, Israel) were connected to anti-leak purple stoppers (28-13 psi, Dubois

Agrinovation product # IS 0340017-B, Quebec, Canada) to ensure that all the emitters stopped at the same time, before being connected to the main line. The overhead emitters (3.2 GPH @60psi, NaanDan Jain product # NET-337, Jalgaon, India) were also connected to the anti-leak purple stopper. Irrigation duration was determined for drip emitters (y=1.16x+8.19, y=water amount (mL), x = time (s)) along with overhead emitters (y = 1.56x+7.07, y = water amount (mL), x = time (s)).

The pressure gauge (Super Pro® Pool Filter 0-60 Pressure Gauge 1/4" Fitting, Moorooka, Australia) helped regulate the SHURflo® Revolution Water Pump (Product # 4008-101-E65 3.0, Pentair, Costa Mesa, CA, USA), when the pressure reached about 50 psi, the pump automatically turns off as per an internal regulatory mechanism in the pump. Pump speed was controlled by a knob and each water tank was also attached to a strainer (Shurflo® (255-313) 1/2" Twist-On Pipe Strainer) to prevent debris from clogging the pump.

Chemicals and Materials. Eleven analytical standard pharmaceuticals: acetaminophen (101.3% purity), caffeine (100.5%), carbamazepine (100%), carbadox (100%), lincomycin (>90%), monensin sodium (90-95%), oxytetracycline (95%), sulfadiazine (99%), sulfamethoxazole (99.2%), trimethoprim (99%), and tylosin (analytical grade) were used (Sigma-Aldrich, St. Louis, MO, USA). Pharmaceuticals were chosen based on varying structures and physiochemical properties as shown in Table 1. Chemicals were dissolved in HPLC-grade methanol (MeOH) to prepare stock solutions at concentrations ranging from 10 to 1000 mg/L. Acetonitrile and anhydrous sodium sulfate (Na_2SO_4) were purchased from EMD Chemicals (Gibbstown, NJ, USA). Ceramic homogenizers, C18 and primary-secondary amine (PSA) were purchased from Agilent Technologies (Santa Clara, CA, USA). Disodium ethylenediaminetetraacetate (Na_2EDTA), formic acid, and sodium chloride (NaCl) were purchased from J.T. Baker (Phillipsburg, NJ, USA). All chemicals used were of analytical grade or better. Oasis® hydrophilic-lipophilic balance (HLB) 6cc (200mg) Extraction Cartridges were purchased from Waters Corporation (Milford, MA, USA).

Soil and Lettuce Information. The soil used was a Michigan loamy sand collected from Charlotte, MI. This soil represents a high water use scenario since sandy soils have lower water holding capacity and loamy sand is a common soil type in California, where much of U.S lettuce is produced. The soil was air-dried for 1–2 weeks and then sieved to 2 mm and stored in a greenhouse. Selected soil properties are provided in Table 2. Nursery pots of 14.6-cm diameter and 10.8-cm height were uniformly packed with the air-dried soil to 9-cm soil depth and approximately 1455 g, resulting in a bulk density of 1.35 g/cm^3.

Concurrently, 4-6 Burpee® Black Seeded Simpson Lettuce (Burpee, Warminster, PA, USA) seeds were planted in sterile potting mix for approximately two weeks before the start of each trial. Burpee® Black Seeded Simpson Lettuce is a commonly grown lettuce type. Seedlings were watered with DI water and Peters Professional® water soluble 20-20-20 general purpose fertilizer (Scotts, Marysville, OH, USA) as needed. After one week, seedlings were thinned and transplanted into individual soil pots. Before transplanting, excess potting mix was separated from transplants and soil pots were saturated with DI water. Plants were left to acclimate for about 2 days before beginning each trial. Soil pots were randomly placed under each emitter.

Overhead irrigated plants had a transparent screen with holes for air flow placed around the pot to minimize water loss from overhead spray. Surface irrigated pots also had a screen in the 30 µg/L trial.

Irrigation Water Preparation. Pharmaceutical stock solutions for Trial 1 (i.e., the 50 µg/L trial) were prepared at 100 mg/L, with the exception of carbadox at 10 mg/L. The stock solutions for Trial 2 (i.e., the 30 µg/L trial) were prepared at 1000 mg/L, except for sulfadiazine at 100 mg/L and carbadox at 10 mg/L. These stock solutions were used to prepare pharmaceutical-spiked irrigation water with the exception of carbadox in Trial 1 and the standards for LC/MS-MS analyses. For Trial 1, carbadox was added to irrigation water from pharmaceutical powder.

Irrigation water for the pharmaceutical water tank in Trial 1 (Figure 2) was prepared at a concentration of 50 µg/L by combining 20 mL of each stock solution (excluding carbadox), 2 mg powdered carbadox, and 39.8 L of DI water. It was assumed that there were no chemical reactions between chemicals in the water tanks. The methanol concentration in the final tank was 0.5%, which would not negatively affect plant growth (Li et al., 2010). Tanks were cleaned out and refilled on 5/3/15 and 5/21/15.

The irrigation water in the pharmaceutical water tank for Trial 2 (Figure 3) was prepared at a concentration of 30 µg/L by combining 1.2 mL of each stock solution (excluding sulfadiazine and carbadox), 12 mL of 100 mg/L sulfadiazine stock solution, 120 mL of 10 mg/L carbadox stock solution, and 39 L of DI water. The methanol concentration in the final tank was 0.357%. Fertilizer was added to the tank water to obtain a final nitrogen concentration of 125 mg/L (i.e., 25 g of 20-20-20 added). Tanks were cleaned out and refilled on 10/2/15 and 10/13/15.

The non-pharmaceutical water tank (i.e., control water tank) contained DI water in Trial 1 and fertilizer solution with 125 mg/L nitrogen in Trial 2. The non-pharmaceutical treatment was used to examine if there was any phytotoxic effect of pharmaceuticals to the lettuce plants, and to obtain matrix background matching water, plant, and soil samples for the LC/MS-MS analysis. The water in both tanks was used to irrigate plants until water level reached the tank outlet, after which the tanks were cleaned out and refilled twice before the end of experiments.

Irrigation Schedule. Plants in Trial 1 were irrigated with 25 to 100 mL of irrigation water each day, and plants in Trial 2 were irrigated with 25 to 125 mL irrigation water. Amounts of irrigation water were determined by using temperature, humidity, soil water content, and evaporation data. Temperature and humidity were measured in the greenhouse, soil water content and evaporation were determined using weight measurements. A more detailed irrigation schedule and irrigation amounts can be found in Tables 3 and 4.

Water Sample Collection and Extraction. Water samples (20 mL) collected from both the pharmaceutical and control tanks were taken daily for Trial 1 and 2–3 times a week during Trial 2 and stored in amber glass vials with polyurethane caps. To extract pharmaceuticals from the water samples, 1 mL of 300 or 3000 mg/L Na_2EDTA was added to each 20 mL sample and vortexed. Although different concentrations of Na_2EDTA were used during the experiments, Na_2EDTA concentration did not greatly affect the recovery of any chemical except for oxytetracycline as indicated by the high relative standard deviation. For the Na_2EDTA and pharmaceutical-water extraction recovery test, 20 mL of both 50-µg/L and 30-µg/L pharmaceutical water was prepared, replicating the experimental irrigation water preparation procedure outlined above. Triplicate irrigation water samples were extracted with both Na_2EDTA concentrations (300 and 3000 mg/L) using the same process as described below. Mass of each pharmaceutical was determined, and percent recovery and relative standard deviation were calculated. Results from this test can be found in Table 5.

HLB cartridges were used with a PrepSep 12-Port Vacuum Manifold (Fischer Scientific, Waltham, MA, USA). The cartridges were conditioned with 5 mL MeOH followed by elution with 5 mL DI water. After conditioning, the water samples were loaded by passing the water sample and Na_2EDTA mixture through the cartridge. The extracted pharmaceuticals were then eluted with 5 mL of MeOH into clean amber vials that were stored in a −20°C freezer until analysis. Extraction procedure was followed from Chuang et al., 2015.

Plant and Soil Sample Preparation. Three overhead and surface irrigated lettuce plants were harvested weekly and control plants were harvested periodically throughout the trials. Shoots were cut at the base of each plant using scissors that were washed with DI water and MeOH between plants. The shoots were washed in 200 mL of DI water. The shoot wash water in Trial 1 was saved for later analysis. Because negligible pharmaceutical residues were washed off the shoots in Trial 1, the shoot wash water in Trial 2 was discarded. Lettuce shoots were dried with a paper towel, weighed, placed in a labeled beaker, and stored in a −20 °C freezer. Next, the soil pots were overturned, and each soil core was cut into 3 vertical soil layers of 3-cm depth using a sterilized knife, referred to as top, middle, and bottom layers. Lettuce roots were separated from each soil layer by washing and removing excess soil. Keeping each soil layer separate, lettuce roots were collected, washed, towel-dried, weighed, and placed in a labeled beaker and held in the −20 °C freezer. The soil from each layer was collected, homogenized, placed in a labeled beaker, and frozen at −20 °C.

After thorough freezing, plants were freeze-dried using a Vitris Sentry freeze mobile lyophilizer (Gardiner, New York, USA), weighed, ground using a Smartgrind electric grinder (Black & Decker, Middleton, WI, USA) or hand-operated mortar and pestle. Ground plant material was stored in foil packets in a freezer until extraction and analysis.

Plant and Soil Extraction Method. Plant extraction was performed following a modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method (Chuang et al., 2015). Initially, 0.25 or 0.50 g of the freeze-dried and ground plant or soil sample was placed into 50-mL centrifuge tubes. After adding 2 mL of 150 mg/L Na_2EDTA, the centrifuge tube was vortexed for 1 minute. Thereafter two ceramic homogenizers and 5 mL of acetronitrile/MeOH (65/35) were added to each tube and vortexed for 1-2 minutes. After adding 2 g Na_2SO_4 and 0.5 g NaCl packets, the tube was vortexed for 1.5 minutes, and centrifuged in a Sorvall® RC 6 Centrifuge for 10 minutes using the size SS-34 rotor at 5000 g, 5 accelerations, and 5 decelerations (Thermo Scientific, Waltham, MA, USA,). Thereafter, 1.3 mL of supernatant was transferred into 1.5 mL centrifuge tubes that were prefilled with 12.5 mg of C18, 12.5 mg of PSA and 225 mg of Na_2SO_4. The small centrifuge tubes were vortexed for 1 minute and then centrifuged in an Eppendorf 5415D centrifuge (Hauppague, NY, USA) for 5 minutes at 10,000 g. Finally, 0.9 mL of supernatant was transferred into a 1 mL amber LC vial containing 0.1 mL of MeOH and stored in the freezer until analysis. Using this procedure pharmaceutical recovery in plant samples ranged from about 72-96% as reported in Chuang et al. (2015).

The extraction procedure for soil samples was similar to the procedure for plant samples after method development and recovery testing. A QuEChERS method was adapted for the soil extraction (Chuang et al., 2015). To compare the pharmaceutical recovery as influenced by Na_2EDTA concentration, 150 mg/L of Na_2EDTA and 300 mg/L of Na_2EDTA were used. Four replicate soil samples (0.25 g each) for each Na_2EDTA treatment were spiked with 0.2 mL of 500 µg/L stock solution (triplicate spiked soil samples were used for lincomycin and pharmaceutical-free soil for sulfamethoxazole because of outliers). The spiked and pharmaceutical-free soils were extracted using the QuEChERS method with either 150 or 300 mg/L of Na_2EDTA with 150 mg/L Na_2EDTA chosen based on recovery between 15% for oxytetracycline and 96% for sulfadiazine as provided in Table 6.

LC-MS/MS Analysis. The extracts were analyzed for pharmaceutical concentrations using a Shimadzu Prominence high performance liquid chromatograph (Colombia, MD, USA) coupled with an Applied Biosystems Sciex QTrap® 4500 triple quadrapole mass spectrometer (Foster City, CA, USA). An Agilent Eclipse Plus C18 Column (2.1 mm × 50 mm, particle size 5 µm) was used for separation. The mobile phase consisted of phase A (0.3% formic acid in DI water) and phase B, acetonitrile/methanol (1/1) with 0.3% formic acid. The flow rate was 0.35 mL/min and the sample injection volume was 10 µL. Pharmaceuticals were quantified using a matrix-based calibration curve. Precursor ions and product ions for qualification and quantification, along with mass spectrometer parameters can be found in Table 7.

Pharmaceutical Concentration and Mass Calculations. Pharmaceutical concentrations in the irrigation water and the amount of pharmaceutical applied to each plant were calculated as follows for all pharmaceuticals except carbadox and oxytetracycline. After LC-MS/MS analysis, the pharmaceutical concentrations in the MeOH extract was divided by 4 to obtain the concentrations in the irrigation water. This was done because during the extraction, the water sample was concentrated by a factor of four. Carbadox and oxytetracycline concentrations in the irrigation water were inaccurate because of the extraction method. For oxytetracycline, Na_2EDTA concentration extraction generally underestimated oxytetracycline. For carbadox, using powder rather than stock solution in the 50-µg/L trial underestimated carbadox after extracting irrigation water. It is unclear why, but the 30-µg/L trial concentrations for carbadox were also inaccurate and much lower than the other pharmaceuticals. Therefore, for both chemicals, the nominal concentrations (i.e., 50 or 30 µg/L) were used. The amount of pharmaceutical applied to each plant was calculated by multiplying the concentrations in the irrigation water by the amount of water applied to each plant on each day.

The pharmaceutical concentrations in the shoot wash water were calculated by dividing LC-MS/MS concentration by eight, because the sample was concentrated by a factor of eight during extraction.

To calculate the pharmaceutical concentrations in the shoots, the concentrations measured by LC-MS/MS were multiplied by 10/9 to obtain the concentrations in the MeOH extract, which were then converted to the concentrations in the dry shoots by multiplying by 0.005 L and then dividing by the dry weight of the shoot sample. The total mass of pharmaceuticals in the shoots was then calculated by multiplying the dry-weight concentrations by the dry weight of the total shoot. Fresh weight concentrations were calculated by dividing the total mass of the pharmaceutical by the total fresh weight of the shoot. Pharmaceutical concentrations in the root were calculated using the same procedure as described for the shoots.

To calculate dry-weight and fresh-weight concentrations and total mass of pharmaceuticals in the soil, the procedure as described above was followed. In Trial 1, the acetaminophen concentration in top soil from one replicate, caffeine concentration in middle soil from one replicate, and lincomycin concentration in bottom soil from one replicate were outliers and excluded from further analysis because their concentrations were at least 10 times greater than the average value. The concentrations from the other two replicates were therefore used. Background pharmaceutical concentration for caffeine, carbamazepine, trimethoprim, oxytetracycline, and tylosin, which was detected in pharmaceutical-free soil samples (Table 6), was subtracted from analyzed soil samples to get final soil pharmaceutical concentration.

Root concentration factors were calculated by dividing the concentration of each pharmaceutical in roots by the concentration in the soil for the same treatment pot at each harvest point. Translocation factors were calculated by dividing the concentration of each pharmaceutical in shoots by the concentration in the roots for the same plant. Each factor was averaged for each treatment for each week and standard deviations were also calculated.

Pharmaceutical mass balance was calculated cumulatively for each week. The amount of pharmaceutical applied was added together for each week. Next, the average mass of pharmaceuticals in the shoots, roots, and soils for each week were combined.

In Trial 2, the pharmaceutical concentrations in the irrigation water were measured every few days, and the average concentrations for the two neighboring samplings and measurements were used for days within that duration. Other calculations were the same as those in Trial 1, except no soil sample measurements were excluded. No samples were corrected for recovery for either trial.

Statistical Analysis. All statistical analyses were conducted using GraphPad PRISM 7. Plant biomass comparisons by trials was analyzed as grouped unpaired t-tests. Statistical significance was determined using the Holm-Sidak method, with alpha = 0.05. Each week was analyzed individually, without assuming a consistent standard deviation. Plant biomass comparisons of each irrigation treatment method within trials were analyzed in the same manner.

RESULTS AND DISCUSSION

Irrigation Water Analysis and Lettuce Biomass. Pharmaceutical concentrations in irrigation water for the 50 and 30 µg/L trials are shown in Figures 2 and 3. While the nominal concentration of all spiked pharmaceuticals was either 50 or 30 µg/L in these two trials, the measured concentrations ranged from 31.06 µg/L to 83.69 µg/L in the 50 µg/L Trial and from

10.65 μg/L to 52.41 μg/L in the 30 μg/L Trial, respectively. The differences between the measured and nominal concentrations might be due to analytical errors or degradation (Chaung et al., 2015).

The difference between the 50and 30-μg/L trial was the starting concentration for pharmaceuticals in irrigation water. Lettuce in the 50-μg/L trial was stunted and showed major necrosis during the experiment (Figure 4), even with periodic fertilizer application. It was determined that growth was limited due to stress because of being exposed to a high concentration of pharmaceutical over most of the growth cycle. For example, previous studies have shown that a decline in plant growth is possible with oxytetracycline exposure (Boxall et al., 2006; Jjemba, 2002). Therefore, for the next trial, plants were given fertilizer throughout the whole growing period and the initial concentration of pharmaceuticals was lowered.

Fresh and dry shoot biomass in the 50 and 30-μg/L trials were measured after each week (Figure 5). A Holm-Sidak two-tailed unpaired t-test ($p < 0.05$) revealed that average dry shoot biomass in the 30-μg/L trial was greater than that harvested in the 50-μg/L trial (Figure 6), likely due to the change in pharmaceutical concentrations of irrigation water and fertilizer application. However, the fresh weight of shoots under overhead irrigation had no significant difference compared to surface irrigated shoots with the exception of week 2 in the 30-μg/L trial for which fresh weight under surface irrigation was greater than that under overhead irrigation ($p = 0.006$).

Pharmaceutical Concentrations in Shoot Wash Water. During harvest in both trials, shoots were washed to simulate consumer washing of lettuce before consumption. Shoot wash water was analyzed for the 50-μg/L trial, but not for the 30-μg/L trial due to low concentrations observed in the first trial (Figure 7); average concentrations for all pharmaceuticals were 2.5

µg/L or below for plants harvested in weeks 2-5. Pharmaceutical concentration data can be found in Table 8 for the 50-µg/L trial.

Pharmaceutical concentrations in the shoot wash water ranged in 0–8.60 µg/L for overhead irrigation and 0–5.37 µg/L for surface irrigation. Greater concentrations of acetaminophen, caffeine, carbamazepine, sulfamethoxazole, trimethoprim, lincomyacin, monensin sodium, and tylosin were observed for 3 or more weeks on overhead irrigated shoots as compared to surface irrigated shoots (Figure 7). Surface-irrigated lettuce concentrations were low compared to overhead-irrigation concentrations because overhead-irrigated lettuce came into direct foliar contact with irrigation water. As water evaporated from lettuce leaves, pharmaceutical residues remaining on lettuce that were removed during washing. These results indicate the importance of consumer processing techniques when handling crops irrigated with reclaimed water. Although concentrations were low in both treatments, improper washing could lead to unintended exposure to some pharmaceuticals.

Oxytetracycline, sulfadiazine, and carbadox concentrations were similar in the shoot wash water regardless of irrigation treatment for 2 or more weeks, differences between pharmaceutical concentrations that were observed were inconclusive because of overlapping standard deviation.

Pharmaceutical Concentrations in Lettuce Shoot. Results for each pharmaceutical concentration in shoots from the 50 and 30-µg/L trials are shown in Figures 8 and 9. For pharmaceuticals of lower lipophilicity, lower molecular weight, and higher water solubility including acetaminophen, caffeine, carbamazepine, sulfadiazine, sulfamethoxazole, carbadox, and oxytetracycline, conclusive difference in their concentrations in the shoot between overhead irrigation and surface irrigation was observed overtime (Figures 8 and 9). Pharmaceutical concentration data can be found in Tables 8 and 9 for the 50-µg/L and 30-µg/L trial, respectively.

However, for pharmaceuticals of high lipophilicity, high molecular weight, and lower water solubility including monensin sodium and tylosin, their shoot concentrations were consistently greater for overhead irrigation as compared to surface irrigation (Figures 8 and 9). Previous studies have also shown that tylosin cannot be taken up by corn, cabbage, and onion, possibly due to its large molecular size (Kang et al., 2013). Therefore, it is likely that the high concentration observed in our experiment was due to overhead irrigation.

There were exceptions for trimethoprim and lincomycin which have a relatively larger molecular weight, higher water solubility, and lower log K_{OW} (Figures 8 and 9). In this case, the trimethoprim concentration in overhead irrigated shoots was greater than that for surface irrigation (Figures 8 and 9), and the lincomycin concentration in overhead irrigated shoots was greater than that for surface irrigation only in the 30 µg/L trial (Figure 9). Irrigation water pH was approximately 7 for both trials. Due to their pK_a values (7.12 and 7.6 respectively), trimethoprim and lincomycin are mostly in neutral and cation form and are likely to either diffuse into the waxy leaf cuticle (Calderón-Preciado et al., 2013) or bind with negatively charged leaf surface under overhead irrigation. It is unclear why this trend did not occur for lincomycin in the 50 µg/L trial, but it is suspected to be due to low biomass and poor plant growth.

Additionally, for pharmaceuticals including acetaminophen, caffeine, carbamazepine, and carbadox, their concentrations in the shoot for the 50 µg/L trial appeared to be greater for overhead than surface irrigation during the first two weeks. However, due to low lettuce biomass, the triplicate shoot samples were pooled, and thus no replicate measurements were available, and no statistical significance could be determined. While most of pharmaceutical concentrations in the shoot did not increase with time, carbamazepine and lincomycin concentrations in the 30 μg/L trial demonstrated an increasing trend over time (Figure 9). This increase overtime indicates that these pharmaceuticals are easily transported into lettuce leaves. Carbamazepine has been detected in leaf tissues and soil pore water at higher concentrations than other nonionic compounds (Goldstein et al. 2014, Carter et al. 2012). Studies have shown that this could be a result of hydrophobicity since carbamazepine has a $\log K_{OW}$ of 2.45. Since carbamazepine is neutral over a range of pH values, it can be translocated easily in plants through mass flow. This means that even if irrigation water has relatively low levels of carbamazepine, it could be possible for plants to more favorably accumulate, but differences occur between plant species (Carvalho et al. 2014). Carbamazepine has also been consistently detected at higher concentrations in pore water and increases overtime in plants. Carter et al. (2014) found that carbamazepine concentration was consistently higher in the pore water (1321−3129 μg/L) over their 40-day experiment. It is not clear why this trend was not observed for the 50 μg/L trial, but it is suspected to be due to low biomass and poor plant growth.

Pharmaceutical Concentrations in Lettuce Root. Results for each pharmaceutical concentration in roots for the 50 and 30-μg/L trials are shown in Figures 10 and 11. Generally, there was no statistically significant difference in pharmaceutical concentration in the roots using the two irrigation treatments based on standard deviation indicating that irrigation method does not play a large role in root accumulation of pharmaceuticals in lettuce. In the 50 μg/L trial, the root concentrations of acetaminophen, carbamazepine, trimethoprim, and tylosin appeared to be greater for overhead as compared to surface irrigated lettuce. However, no such differences were observed in the 30-μg/L trial. It was speculated that the difference observed between trials was likely due to differences in growing conditions. Also, due to the low root biomass in the 50-µg/L trial, all of the roots collected from the three pots were combined. Consequently, no statistical significance can be inferred. Pharmaceutical concentration data can be found in Tables 8 and 9 for the 50-µg/L and 30-µg/L trial, respectively.

In surface-irrigated plants, chemicals are taken up via soil pore water (Boxall et al., 2006). Root uptake of organic chemicals is related to the K_{ow} of the chemical and uptake into roots is greater for hydrophobic compounds, whereas polar compounds are accumulated less. Root concentration trends were observed in the 30 µg/L trial overtime regardless of irrigation method (Figure 11). There was an increase in root concentration overtime for carbamazepine, sulfadiazine, carbadox, trimethoprim, lincomycin, and oxytetracycline, indicating that these pharmaceuticals are able to be taken up by lettuce roots. In a study using alfalfa, oxytetracycline uptake by roots was concluded as being both an active and passive process, treated alfalfa had a liner increase in oxytetracycline uptake over 4 hours (Kong et al. 2007).

There was a decrease in root concentration overtime for acetaminophen. This result is supported by a hydroponic study by Bartha et al. (2010), which found higher concentrations of acetaminophen roots than shoots after 24 hours, however a steep decrease in both tissues concentrations was seen after one week. Caffeine, sulfamethoxazole, monensin sodium, and tylosin had relatively stable root concentrations overtime.

Pharmaceutical Concentrations in Soil. Results for each pharmaceutical concentration in the soil layers for the 50 and 30-µg/L trials are shown in Figures 12 and 13. All pharmaceutical concentrations in soil were similar regardless of irrigation method in either trial, which was expected since soil was not covered for overhead-irrigated lettuce (i.e., simulating field irrigation conditions). Thus, soil was exposed to pharmaceutical irrigation water in both treatments. Pharmaceutical concentration data can be found in Tables 8 and 9 for the 50-µg/L and 30-µg/L trial, respectively.

Caffeine, sulfadiazine, sulfamethoxazole, lincomycin, oxytetracycline and monensin sodium showed no patterns in concentrations between the different layers. Sulfadiazine has been shown to dissipate in soil. When Boxall et al. (2006) used a loamy sand soil spiked with pharmaceuticals, 50% of the sulfadiazine was dissipated in the soil in less than 103 days, and 90% by 103 days. Wang et al. (2012) found that cation exchange was the primary mechanism for lincomycin sorption into soil. Since it competes with potassium and calcium for cation exchange sites, having these ions in solution decreases lincomycin sorption. There is also less sorption of lincomycin in soil that have a lower cation exchange capacity. Thus, lincomycin was able to leach downward and was evenly distributed.

Acetaminophen concentration was lower in soil compared to the other pharmaceuticals in the 50-µg/L trial and was not detected in the 30-µg/L trial. Because of its low $\log K_{OW}$ (0.46), partitioning into soil organic matter is predicted to be minor. Therefore, it was likely that acetaminophen was degraded in soil.

Carbamazepine, trimethoprim, and tylosin concentrations increased in the top layer of soil overtime in the 50-µg/L trial (Figure 12), in addition to carbadox in the 30- µg/L trial (Figure 13), suggesting strong sorption to soils. It is unclear why a different trend was observed for carbadox between trials. Carbamazepine has been reported as being persistent in soil, with dissipation reported over 40 days (Carter et al. 2014).

Pharmaceutical Root Concentration Factors and Translocation Factors. Pharmaceutical root concentration factors and translocation factors can be found in Table 10 for the 30-µg/L trial. Factors were not calculated for the 50-µg/L trial since samples had to be combined before extractions because of low biomass and because plants were unhealthy compared to 30-µg/L trial plants. Root concentration factors were not available when pharmaceuticals were not detected in soils as seen with acetaminophen over the whole growing period (weeks 1-5) and trimethoprim for weeks 1-3. Root concentration factors for all pharmaceuticals was similar between treatments for most weeks. This was expected since all plants received the same mass of pharmaceuticals each week regardless of treatment and because soil was not covered for overhead-irrigated lettuce, allowing for pharmaceutical contaminated water to reach soils and be taken up through lettuce roots.

Translocation factors below 1 generally indicate that pharmaceuticals are not readily transported from roots to shoots in plants (Eggen et al., 2012). Translocation factors above 1 generally indicate that pharmaceuticals are readily transported from roots to shoots and can accumulate in shoots (Eggen et al., 2012). Pharmaceuticals that did not show a difference in concentration between irrigation methods had similar translocation factors for both overhead-irrigated and surface-irrigated lettuce overtime (acetaminophen, caffeine, carbamazepine, sulfadiazine, sulfamethoxazole, carbadox, and oxytetracycline), meaning irrigation method does not play a large role in pharmaceutical accumulation overtime. Carbamazepine had a consistently high translocation factor (>1) for both treatments indicating that it can be easily transported from lettuce roots to shoots and can also accumulate in plants, supporting previous conclusions on pharmaceutical concentration and trends overtime.

Trimethoprim and lincomycin showed differences in translocation factors between treatments for weeks 1 and 2, with overhead-irrigated lettuce having higher concentration factors than surface-irrigated. While surface-irrigated lettuce translocation factors clearly indicate a transfer of pharmaceuticals from roots to shoots, overhead-irrigated lettuce translocation factors do not because of direct foliar contact between pharmaceutical contaminated water and lettuce shoots. Differences between the two translocation factors can indicate that surface-irrigated plants have a lower transference from roots to shoots than overhead-irrigated because of the direct contact. Both pharmaceuticals had higher concentrations in overhead-irrigated lettuce than surface-irrigated lettuce. Because their translocation factors started out as different and converged overtime, we can conclude that irrigation method can play a difference in pharmaceutical concentration, but because both pharmaceuticals are also more easily transported from roots to shoots because of water solubility and molecular weight, the differences between irrigation methods can diminish overtime.

Monensin sodium and tylosin both had much higher translocation factors in overhead-irrigated lettuce than surface-irrigated. This difference indicates that these pharmaceuticals are not readily transferred from roots to shoots in lettuce, but that overhead irrigation can result in higher transference to shoots because of foliar contact. These results support the previous conclusions that overhead-irrigated lettuce will have higher concentrations of these pharmaceuticals than surface-irrigated.

Pharmaceutical Mass Balance. Pharmaceutical mass balance information can be found in Tables 11 and 12 for the 50-µg/L and 30-µg/L trials, respectively. Acetaminophen, caffeine, carbamazepine, and oxytetracycline all had a higher mass in shoots compared to roots for both treatments. Trimethoprim, monensin sodium, and tylosin had a higher mass in overhead-irrigated shoots than roots for both trials, but no difference was seen between roots and shoots in surface-irrigated samples. Sulfamethoxazole and trimethoprim also had a higher mass in overhead-irrigated shoots than roots, but only for the 50-µg/L trial and lincomycin had the same trend except in the 30-µg/L trial, indicating that pharmaceuticals can transfer to the edible portion of lettuce and could lead to human exposure through consumption.

Monensin sodium and tylosin had higher mass in overhead compared to surface-irrigated shoots for all weeks during both trials. Sulfamethoxazole, lincomycin, and oxytetracycline showed the same trend, but just in one trial; the 50-µg/L trial for sulfamethoxazole and 30-µg/L for the other two pharmaceuticals, indicating that irrigation method can play a role in final pharmaceutical levels in lettuce shoots.

Carbamazepine, monensin sodium, and tylosin had increasing mass in shoots overtime in both trials: carbamazepine in both irrigation treatments, and monensin sodium and tylosin in the overhead irrigated lettuce. Carbadox showed the same trend in the 30-µg/L trial, indicating that shoots can accumulate pharmaceuticals overtime, and harvest time could play a role in final levels of pharmaceuticals in lettuce.

Sulfadiazine had very low mass in roots and shoots for both trials and sulfamethoxazole in the 30-µg/L trial. Oxytetracycline had a low amount in roots for the 50-µg/L trial, whereas, carbamazepine increased in roots overtime in the 30-µg/L trial.

All pharmaceuticals had a higher mass in soil compared to shoots or roots in both trials for a majority of weeks with the exception of acetaminophen and oxytetracycline in the 30-µg/L trial, indicating that soil sorption can be significant and pharmaceuticals can be persistent in soils (Boxall et al., 2006). The time needed to dissipate the oxytetracycline level by 50% in soil was less than 103 days and by 90% was greater than 152 days (Boxall et al., 2006). Our finding is in agreement with other studies showing that oxytetracycline sorbs strongly to soils (Christian et al., 2003). Tetracyclines can form complexes with cations, like calcium, in soil and partition into soil organic matter. The oxytetracycline level in soil was likely low because of the low recovery and soil recovery method (Table 6.), so final amounts reported may be underestimated. Acetaminophen was not detected in soils in the 30-µg/L trial. Trimethoprim, tylosin and carbamazepine had an increased mass in soil overtime in the 50-µg/L trial and carbamazepine also exhibited the same trend in the 30-µg/L trial indicating that these pharmaceuticals can sorb to soils. Tylosin has been reported to dissipate in soils by 50and 90% in less than 103 and 103 days respectively (Boxall et al., 2006).

Pharmaceutical recovery percentages for both trials can be found in Figures 14 and 15. Carbamazepine had the highest recovery in both trials (over 40%) among all weeks. All other pharmaceuticals had low overall recovery, possibly due to plant metabolism, bacterial metabolism, photodegradation, or irreversible soil sorption. Taking acetaminophen as an example, Bartha et al. (2010) found that uptake and concentration of acetaminophen increased in the first 72 hours, but decreased thereafter. The highest concentrations of acetaminophen were found in roots and leaves after 24 hours. Due to the decrease after week 1 of acetaminophen treatment, the authors suggest the presence of both a plant independent pathway for acetaminophen sorption or degradation and a plant dependent acetaminophen metabolism pathway. Their LC-MS/MS analysis also detected two acetaminophen metabolites in plant tissues, indicating that metabolism could play a large role in low recovery of acetaminophen as seen in both trials. Other pharmaceuticals, such as caffeine, have been shown to degrade through photolysis in laboratory experiments (Bruton et al., 2011)

IMPLICATIONS

Our findings may have interesting implications on utilizing reclaimed water to irrigate vegetable crops. Despite the wide use of overhead sprinkler systems, their use in vegetable production should be discouraged when using reclaimed water for irrigation due to greater concentration and mass of some pharmaceuticals (specifically monensin sodium and tylosin) in overhead as opposed to surface-irrigated lettuce shoots. Our study showed the ubiquitous accumulation of pharmaceuticals in lettuce from irrigation water, demonstrating the need for further assessing the environmental and food safety risks associated with using pharmaceutical-contaminated water for irrigation.

APPENDIX

Table 1. Physicochemical properties of pharmaceuticals used in this study (Chuang et al., 2015).

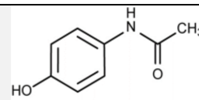
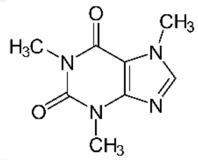
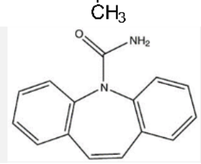
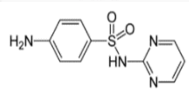
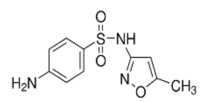
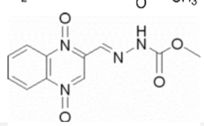
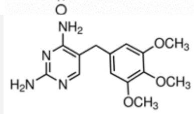
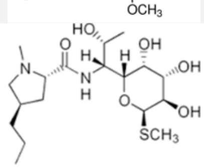
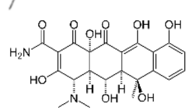
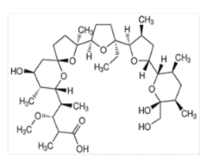
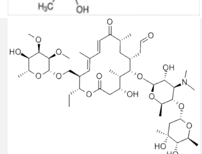
Pharmaceutical	Molecular Weight (g/mol)	Chemical Structure	Water Solubility (mg/L)	p*K*a	$\log K_{OW}$
acetaminophen	151.16		14000	9.38	0.46
caffeine	194.19		21600	10.4	-0.07
carbamazepine	236.27		18	2.3, 13.9	2.45
sulfadiazine	250.28		77	2.01, 6.99	-0.09
sulfamethoxazole	253.25		610	1.6, 5.7	0.89
carbadox	262.22		1755	1.8, 10.5	-1.22
trimethoprim	290.32		400	7.12	0.91
lincomycin	406.54		927	7.6	0.2
oxytetracycline	460.43		313	3.57, 7.49, 9.44	-0.9
monensin sodium	692.87		Slightly Soluble	4.3	5.43
tylosin	916.10		5	7.73	3.27

Table 2. Soil properties for Michigan loamy sand used in this study.

Sand	81.3%
Silt	10.5%
Clay	8.2%
Soil pH	7.4
Phosphorus	71 mg/kg
Potassium	50 mg/kg
Magnesium	126 mg/kg
Calcium	1298 mg/kg
Cation Exchange Capacity	7.0 meq/100 g
Organic Matter	2.5%

Table 3. Irrigation schedule and water volume in the 50-µg/L Trial.

Date	Irrigation Amounts (mL)	Notes
04/20/15	50	
04/21/15	25	
04/22/15	50	
04/23/15	50	
04/24/15	75	
04/25/15	75	
04/26/15	75	
04/27/15	75	Plants harvested this date received 25 mL of irrigation water before harvest.
04/28/15	75	
04/29/15	75	
04/30/15	75	
05/01/15	75	
05/02/15	75	
05/03/15	75	New pharmaceutical tank water (50 µg/L)
05/04/15	75	Plants harvested this date received 25 mL of irrigation water before harvest
05/05/15	50	
05/06/15	50	
05/07/15	75	
05/08/15	75	
05/09/15	75	
05/10/15	75	
05/11/15	75	Plants harvested this date received 25 mL of irrigation water before harvest
05/12/15	75	
05/13/15	75	
05/14/15	75	
05/15/15	75	
05/16/15	75	
05/17/15	75	
05/18/15	75	Plants harvested this date received 25 mL of irrigation water before harvest
05/19/15	75	
05/20/15	75	
05/21/15	100	New pharmaceutical tank water (50 µg/L)
05/22/15	100	
05/23/15	100	
05/24/15	100	
05/25/15	25	Plants harvested this date received 25 mL of irrigation water before harvest
04/20/15	50	

Table 4. Irrigation schedule and water volume in the 30-µg/L Trial.

Date	Irrigation Amounts (mL)	Notes
09/17/15	25	
09/18/15	50	
09/19/15	75	
09/20/15	75	
09/21/15	75	
09/22/15	75	
09/23/15	100	
09/24/15	100	Plants harvested this date received 25 mL of irrigation water before harvest
09/25/15	125	
09/26/15	125	
09/27/15	125	
09/28/15	100	
09/29/15	100	
09/30/15	100	
10/01/15	100	Plants harvested this date received 25 mL of irrigation water before harvest
10/02/15	150	New pharmaceutical tank water (30 µg/L)
10/03/15	125	
10/04/15	125	
10/05/15	125	
10/06/15	100	
10/07/15	100	
10/08/15	100	Plants harvested this date received 25 mL of irrigation water before harvest
10/09/15	100	
10/10/15	100	
10/11/15	100	
10/12/15	125	
10/13/15	150	New pharmaceutical tank water (30 µg/L)
10/14/15	125	
10/15/15	125	Plants harvested this date received 25 mL of irrigation water before harvest
10/16/15	125	
10/17/15	125	
10/18/15	125	New pharmaceutical tank water (30 µg/L)
10/19/15	125	
10/20/15	125	
10/21/15	125	
10/22/15	25	Plants harvested this date received 25 mL of irrigation water before harvest

Table 5. The effect of Na_2EDTA concentrations on the pharmaceutical extractions from water.

Pharmaceutical	Pharmaceutical water concentration (µg/L)	Concentration of Na_2EDTA (mg/L)	Percent Recovery of Pharmaceutical (%)	Relative Standard Deviation (%)
Acetaminophen	50	3000	102.9	0.812
Acetaminophen	50	300	105.5	2.577
Acetaminophen	30	3000	118.8	1.582
Acetaminophen	30	300	113.8	4.159
Caffeine	50	3000	101.9	0.881
Caffeine	50	300	109.6	2.542
Caffeine	30	3000	121.4	5.166
Caffeine	30	300	121.3	2.717
Carbamazepine	50	3000	113.0	1.175
Carbamazepine	50	300	113.1	1.990
Carbamazepine	30	3000	143.1	2.813
Carbamazepine	30	300	135.5	1.227
Sulfadiazine	50	3000	105.1	1.209
Sulfadiazine	50	300	104.8	3.741
Sulfadiazine	30	3000	126.5	3.015
Sulfadiazine	30	300	117.9	2.887
Sulfamethoxazole	50	3000	107.1	1.124
Sulfamethoxazole	50	300	102.6	4.764
Sulfamethoxazole	30	3000	115.7	3.930
Sulfamethoxazole	30	300	107.0	3.077
Carbadox	50	3000	121.6	1.480
Carbadox	50	300	107.9	7.012
Carbadox	30	3000	130.6	5.021
Carbadox	30	300	120.6	4.048
Trimethoprim	50	3000	104.0	2.059
Trimethoprim	50	300	107.0	6.553
Trimethoprim	30	3000	122.7	4.275
Trimethoprim	30	300	117.2	5.649
Lincomycin	50	3000	110.2	0.930
Lincomycin	50	300	109.4	3.310
Lincomycin	30	3000	133.3	3.353
Lincomycin	30	300	131.1	1.459
Oxytetracycline	50	3000	81.1	17.746
Oxytetracycline	50	300	77.0	9.267
Oxytetracycline	30	3000	75.8	52.486
Oxytetracycline	30	300	82.8	6.013
Monensin Sodium	50	3000	88.4	2.421
Monensin Sodium	50	300	96.8	1.729
Monensin Sodium	30	3000	114.6	6.321
Monensin Sodium	30	300	110.2	5.692
Tylosin	50	3000	93.4	1.529
Tylosin	50	300	94.2	3.973
Tylosin	30	3000	101.4	5.223
Tylosin	30	300	94.0	3.045

Table 6. Pharmaceutical recovery in spiked and pharmaceutical-free soil extracted with 150 mg/L Na_2EDTA.

Pharmaceutical	Percent Recovery in spiked soil (%)	Relative Standard Deviation in spiked soil (%)	Average concentration in pharmaceutical-free soil samples (μg/L)
Acetaminophen	84.7	3.981	N/A
Caffeine	76.7	7.563	0.3762
Carbamazepine	86.3	4.630	0.0106
Sulfadiazine	85.3	9.419	N/A
Sulfamethoxazole*	76.2	4.278	N/A
Carbadox	92.7	3.750	N/A
Trimethoprim	90.2	3.760	0.0387
Lincomycin**	83.7	4.483	N/A
Oxytetracycline	16.1	3.704	0.2879
Monensin Sodium	77.7	2.348	N/A
Tylosin	80.0	2.234	0.3025

* Triplicate used for pharmaceutical-free soil

** Triplicate used for spiked soil

Table 7. Precursor ions, product ions, and mass spectrometer parameters used in qualification and quantification of pharmaceuticals.

Pharmaceutical	Precursor Ion (m/z)	Product Ion (m/z)	DP (V)	EP (V)	CE (V)	CXP (V)
Acetaminophen	151.931	110	60	10	20	8
		93	60	10	30	6
Caffeine	194.975	138	60	10	30	10
		110	60	10	30	6
Carbamazepine	236.961	193.7	80	10	30	10
		192	80	10	30	12
Sulfadiazine	250.907	156	60	10	20	12
		108	60	10	30	8
Sulfamethoxazole	253.958	155.9	60	10	20	8
		107.9	60	10	30	6
Carbadox	262.92	230.9	60	10	20	12
		144.8	60	10	30	8
Trimethoprim	291.044	261	80	10	30	12
		230	100	10	30	12
Lincomycin	407.122	126	60	10	30	8
		359.1	80	10	30	6
Oxytetracycline	460.982	426.1	60	10	30	8
		283.1	60	10	50	8
Monensin Sodium	694.227	676.3	120	10	50	6
		480	120	10	70	6
Tylosin	916.323	173.8	100	10	40	10
		83	60	10	100	4

Curtain gas (psi) = 20
ionspray voltage (V) = 5000
ion source temperature = 700
ion source gas pressure = 60 & 90
Precursor ion is used for qualification and product ions are used for quantification

Table 8. Pharmaceutical concentrations for overhead and surface irrigated plants in Trial 1 (nominal pharmaceutical concentrations of 50 µg/L in irrigation water).

Pharmaceutical	Sample Type	Harvest Week	Average Overhead Concentration (µg/kg)	Standard Deviation (µg/kg)	Average Surface Concentration (µg/kg)	Standard Deviation (µg/kg)
Acetaminophen	Shoot Wash	1	1.034	1.069	N/A	N/A
	Shoot Wash	2	0.750	0.475	N/A	N/A
	Shoot Wash	3	0.923	0.082	0.036	N/A
	Shoot Wash	4	0.872	0.510	0.178	N/A
	Shoot Wash	5	0.839	0.716	0.134	0.171
	Shoot	1	99.803	N/A	111.049	N/A
	Shoot	2	17.918	N/A	41.769	N/A
	Shoot	3	16.877	8.361	19.152	6.506
	Shoot	4	18.922	15.411	14.240	5.048
	Shoot	5	29.428	16.488	18.624	8.355
	Root	1	1.977	N/A	0.000	N/A
	Root	2	49.383	N/A	7.580	N/A
	Root	3	31.139	N/A	24.091	N/A
	Root	4	39.377	N/A	13.614	N/A
	Root	5	40.583	N/A	0.000	N/A
	Soil - Top Layer	1	5.241	2.993	8.598	N/A
	Soil - Top Layer	2	0.542	N/A	2.264	1.454
	Soil - Top Layer	3	5.324	N/A	5.206	N/A
	Soil - Top Layer	4	6.691	4.667	4.177	2.495
	Soil - Top Layer	5	7.993	1.652	5.740	4.048
	Soil - Middle Layer	1	3.118	2.441	1.023	0.097
	Soil - Middle Layer	2	4.284	N/A	5.148	1.908
	Soil - Middle Layer	3	3.752	2.202	1.340	1.044
	Soil - Middle Layer	4	4.495	2.722	1.409	N/A
	Soil - Middle Layer	5	3.410	1.331	1.392	N/A
	Soil - Bottom Layer	1	5.123	N/A	2.470	N/A
	Soil - Bottom Layer	2	4.875	1.542	2.463	2.874
	Soil - Bottom Layer	3	6.107	3.422	4.215	5.309
	Soil - Bottom Layer	4	2.976	2.942	1.238	0.984
	Soil - Bottom Layer	5	0.407	N/A	3.664	1.535
Caffeine	Shoot Wash	1	8.597	12.436	5.374	7.494
	Shoot Wash	2	1.013	0.444	N/A	N/A
	Shoot Wash	3	1.354	0.248	0.026	N/A
	Shoot Wash	4	1.310	0.342	N/A	N/A
	Shoot Wash	5	1.139	0.862	0.055	N/A
	Shoot	1	104.606	N/A	57.346	N/A
	Shoot	2	143.374	N/A	11.037	N/A
	Shoot	3	65.443	46.750	121.271	56.514
	Shoot	4	70.595	76.561	62.257	52.063
	Shoot	5	74.691	43.123	58.465	71.643
	Root	1	668.121	N/A	229.720	N/A
	Root	2	27.862	N/A	100.341	N/A
	Root	3	67.759	N/A	126.059	N/A
	Root	4	178.860	N/A	41.603	N/A
	Root	5	16.626	N/A	365.954	N/A
	Soil - Top Layer	1	30.847	34.148	80.056	56.564
	Soil - Top Layer	2	69.952	92.526	41.720	45.862
	Soil - Top Layer	3	24.805	6.059	35.166	10.765
	Soil - Top Layer	4	33.322	23.203	34.931	11.659
	Soil - Top Layer	5	24.248	17.610	112.559	164.666
	Soil - Middle Layer	1	1.559	1.680	14.487	14.538
	Soil - Middle Layer	2	5.072	7.173	10.476	9.865
	Soil - Middle Layer	3	28.274	5.925	25.020	23.235
	Soil - Middle Layer	4	52.408	51.954	40.055	22.553

Table 8. (cont'd)

	Soil - Middle Layer	5	18.114	4.153	27.655	6.389
	Soil - Bottom Layer	1	14.259	12.442	7.390	9.143
	Soil - Bottom Layer	2	8.710	11.814	12.743	7.288
	Soil - Bottom Layer	3	71.045	102.470	31.398	19.887
	Soil - Bottom Layer	4	13.364	10.031	23.439	1.240
	Soil - Bottom Layer	5	19.038	15.104	2.415	2.634
Carbamazepine	Shoot Wash	1	2.082	0.801	0.039	0.010
	Shoot Wash	2	1.834	0.574	0.094	0.105
	Shoot Wash	3	2.103	0.293	0.144	0.083
	Shoot Wash	4	2.327	0.680	0.200	0.068
	Shoot Wash	5	1.638	0.864	0.208	0.057
	Shoot	1	71.273	N/A	28.495	N/A
	Shoot	2	274.891	N/A	40.085	N/A
	Shoot	3	140.603	108.492	179.412	168.385
	Shoot	4	86.226	38.494	152.191	119.655
	Shoot	5	152.127	32.282	159.028	43.062
	Root	1	1068.001	N/A	26.065	N/A
	Root	2	142.020	N/A	9.832	N/A
	Root	3	54.251	N/A	7.101	N/A
	Root	4	169.495	N/A	42.218	N/A
	Root	5	20.492	N/A	26.896	N/A
	Soil - Top Layer	1	8.783	5.315	104.912	127.703
	Soil - Top Layer	2	67.585	62.281	34.945	15.217
	Soil - Top Layer	3	53.952	18.728	65.609	23.308
	Soil - Top Layer	4	86.441	23.076	109.056	21.822
	Soil - Top Layer	5	136.472	25.845	132.407	7.640
	Soil - Middle Layer	1	12.151	21.046	0.000	0.000
	Soil - Middle Layer	2	9.234	7.563	11.105	12.480
	Soil - Middle Layer	3	21.989	5.905	19.089	9.219
	Soil - Middle Layer	4	30.689	16.272	17.141	9.018
	Soil - Middle Layer	5	18.528	2.891	17.664	4.847
	Soil - Bottom Layer	1	0.000	0.000	3.307	5.728
	Soil - Bottom Layer	2	0.000	0.000	6.806	11.788
	Soil - Bottom Layer	3	6.820	11.813	2.721	3.427
	Soil - Bottom Layer	4	7.513	10.964	5.107	4.432
	Soil - Bottom Layer	5	21.291	11.286	39.762	44.714
Sulfadiazine	Shoot Wash	1	5.501	7.072	1.399	1.758
	Shoot Wash	2	0.715	0.356	N/A	N/A
	Shoot Wash	3	0.813	0.085	N/A	N/A
	Shoot Wash	4	0.514	0.128	N/A	N/A
	Shoot Wash	5	0.179	0.222	0.123	N/A
	Shoot	1	0.000	N/A	0.000	N/A
	Shoot	2	0.000	N/A	0.000	N/A
	Shoot	3	0.040	0.070	0.334	0.578
	Shoot	4	0.317	0.496	0.000	0.000
	Shoot	5	0.019	0.033	1.595	2.762
	Root	1	0.000	N/A	1.355	N/A
	Root	2	0.235	N/A	0.000	N/A
	Root	3	0.964	N/A	0.383	N/A
	Root	4	1.550	N/A	0.696	N/A
	Root	5	0.595	N/A	0.768	N/A
	Soil - Top Layer	1	0.396	0.686	2.120	1.934
	Soil - Top Layer	2	1.929	3.082	0.258	0.299
	Soil - Top Layer	3	1.548	2.046	0.683	0.594
	Soil - Top Layer	4	5.058	0.501	3.001	0.727
	Soil - Top Layer	5	1.275	0.774	3.249	3.445
	Soil - Middle Layer	1	0.035	0.060	0.941	1.630
	Soil - Middle Layer	2	0.000	0.000	0.000	0.000
	Soil - Middle Layer	3	0.235	0.407	1.551	1.677
	Soil - Middle Layer	4	1.936	2.861	10.115	15.927
	Soil - Middle Layer	5	0.446	0.773	0.744	1.161

Table 8. (cont'd)

	Soil - Bottom Layer	1	5.342	9.253	0.000	0.000
	Soil - Bottom Layer	2	0.000	0.000	0.145	0.251
	Soil - Bottom Layer	3	0.000	0.000	3.537	1.830
	Soil - Bottom Layer	4	0.233	0.403	1.715	2.618
	Soil - Bottom Layer	5	0.000	0.000	0.000	0.000
Sulfamethoxazole	Shoot Wash	1	1.195	0.326	0.099	0.054
	Shoot Wash	2	0.805	0.381	N/A	N/A
	Shoot Wash	3	1.012	0.075	0.703	N/A
	Shoot Wash	4	0.708	0.204	0.002	N/A
	Shoot Wash	5	0.336	0.281	0.011	N/A
	Shoot	1	34.561	N/A	3.350	N/A
	Shoot	2	1.801	N/A	0.000	N/A
	Shoot	3	37.461	64.884	30.214	52.332
	Shoot	4	1.279	2.216	0.557	0.964
	Shoot	5	5.118	8.864	0.000	0.000
	Root	1	60.340	N/A	19.635	N/A
	Root	2	12.151	N/A	6.225	N/A
	Root	3	4.467	N/A	3.612	N/A
	Root	4	36.767	N/A	1.295	N/A
	Root	5	2.918	N/A	8.768	N/A
	Soil - Top Layer	1	11.570	20.040	0.000	0.000
	Soil - Top Layer	2	0.000	0.000	76.496	132.495
	Soil - Top Layer	3	0.000	0.000	0.000	0.000
	Soil - Top Layer	4	45.595	61.571	0.940	1.628
	Soil - Top Layer	5	0.000	0.000	41.065	71.127
	Soil - Middle Layer	1	0.000	0.000	0.000	0.000
	Soil - Middle Layer	2	0.000	0.000	0.000	0.000
	Soil - Middle Layer	3	0.000	0.000	13.407	23.221
	Soil - Middle Layer	4	2.780	4.815	18.635	32.277
	Soil - Middle Layer	5	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	1	0.005	0.008	0.000	0.000
	Soil - Bottom Layer	2	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	3	57.763	100.049	0.000	0.000
	Soil - Bottom Layer	4	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	5	75.659	131.045	0.000	0.000
Carbadox	Shoot Wash	1	0.605	N/A	N/A	N/A
	Shoot Wash	2	0.083	N/A	N/A	N/A
	Shoot Wash	3	N/A	N/A	N/A	N/A
	Shoot Wash	4	N/A	N/A	N/A	N/A
	Shoot Wash	5	N/A	N/A	N/A	N/A
	Shoot	1	11.505	N/A	7.708	N/A
	Shoot	2	2.526	N/A	0.000	N/A
	Shoot	3	1.339	1.760	10.891	11.213
	Shoot	4	0.000	0.000	0.000	0.000
	Shoot	5	0.000	0.000	0.000	0.000
	Root	1	41.103	N/A	32.856	N/A
	Root	2	3.716	N/A	4.717	N/A
	Root	3	4.203	N/A	5.385	N/A
	Root	4	5.047	N/A	0.000	N/A
	Root	5	16.178	N/A	7.817	N/A
	Soil - Top Layer	1	0.000	0.000	1.309	1.683
	Soil - Top Layer	2	1.282	1.829	0.000	0.000
	Soil - Top Layer	3	0.000	0.000	2.839	2.465
	Soil - Top Layer	4	3.679	3.187	4.871	3.320
	Soil - Top Layer	5	1.683	2.915	4.446	2.522
	Soil - Middle Layer	1	0.000	0.000	0.092	0.160
	Soil - Middle Layer	2	0.000	0.000	0.000	0.000
	Soil - Middle Layer	3	0.000	0.000	0.000	0.000
	Soil - Middle Layer	4	39.320	49.058	0.000	0.000
	Soil - Middle Layer	5	1.113	1.928	0.000	0.000
	Soil - Bottom Layer	1	3.200	5.542	0.000	0.000

Table 8. (cont'd)

	Soil - Bottom Layer	2	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	3	0.000	0.000	2.869	4.969
	Soil - Bottom Layer	4	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	5	0.000	0.000	0.000	0.000
Trimethoprim	Shoot Wash	1	2.422	2.828	1.126	N/A
	Shoot Wash	2	0.740	0.206	N/A	N/A
	Shoot Wash	3	1.265	0.106	0.050	N/A
	Shoot Wash	4	1.490	0.155	N/A	N/A
	Shoot Wash	5	1.240	0.690	N/A	N/A
	Shoot	1	88.132	N/A	1.416	N/A
	Shoot	2	33.520	N/A	1.096	N/A
	Shoot	3	60.262	27.379	10.469	10.997
	Shoot	4	33.429	25.903	2.241	0.689
	Shoot	5	30.915	13.131	1.302	0.761
	Root	1	15.935	N/A	9.781	N/A
	Root	2	18.662	N/A	6.984	N/A
	Root	3	16.688	N/A	5.473	N/A
	Root	4	24.421	N/A	5.446	N/A
	Root	5	23.027	N/A	14.167	N/A
	Soil - Top Layer	1	5.383	2.233	14.921	3.834
	Soil - Top Layer	2	17.377	6.854	21.402	4.767
	Soil - Top Layer	3	17.062	5.148	40.424	15.787
	Soil - Top Layer	4	35.308	10.048	94.069	62.678
	Soil - Top Layer	5	60.885	12.821	102.634	23.960
	Soil - Middle Layer	1	0.725	0.825	0.000	0.000
	Soil - Middle Layer	2	2.890	3.160	3.944	2.838
	Soil - Middle Layer	3	3.250	2.780	10.641	6.479
	Soil - Middle Layer	4	6.655	5.413	4.250	3.774
	Soil - Middle Layer	5	55.636	90.641	5.785	4.942
	Soil - Bottom Layer	1	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	2	0.000	0.000	0.889	1.540
	Soil - Bottom Layer	3	0.981	1.700	0.631	0.979
	Soil - Bottom Layer	4	0.836	1.448	4.680	6.633
	Soil - Bottom Layer	5	4.233	5.617	8.660	10.968
Lincomycin	Shoot Wash	1	0.224	0.152	0.147	N/A
	Shoot Wash	2	0.278	0.291	N/A	N/A
	Shoot Wash	3	1.045	0.103	0.010	N/A
	Shoot Wash	4	0.778	0.108	N/A	N/A
	Shoot Wash	5	0.739	0.483	N/A	N/A
	Shoot	1	24.739	N/A	61.012	N/A
	Shoot	2	35.321	N/A	4.675	N/A
	Shoot	3	171.134	206.070	324.612	252.785
	Shoot	4	18.546	6.461	23.161	37.021
	Shoot	5	12.298	3.880	5.916	9.027
	Root	1	41.944	N/A	351.732	N/A
	Root	2	532.451	N/A	1092.944	N/A
	Root	3	15.839	N/A	107.499	N/A
	Root	4	181.828	N/A	15.878	N/A
	Root	5	14.728	N/A	13.729	N/A
	Soil - Top Layer	1	25.435	24.585	24.902	15.922
	Soil - Top Layer	2	41.480	21.230	11.917	2.871
	Soil - Top Layer	3	10.298	3.430	14.558	4.800
	Soil - Top Layer	4	22.144	7.289	184.537	296.013
	Soil - Top Layer	5	5.865	2.456	12.461	4.214
	Soil - Middle Layer	1	4.865	0.638	39.692	58.747
	Soil - Middle Layer	2	10.142	5.321	6.730	2.057
	Soil - Middle Layer	3	7.430	3.164	18.459	18.659
	Soil - Middle Layer	4	43.944	68.189	7.147	1.455
	Soil - Middle Layer	5	4.182	1.552	10.599	8.181
	Soil - Bottom Layer	1	3.963	1.118	21.310	31.733
	Soil - Bottom Layer	2	4.561	2.563	38.414	25.766

Table 8. (cont'd)

	Soil - Bottom Layer	3	7.651	3.191	4.871	0.882
	Soil - Bottom Layer	4	10.276	9.279	4.583	1.930
	Soil - Bottom Layer	5	3.190	1.173	6.342	5.475
Oxytetracycline	Shoot Wash	1	0.085	0.029	0.105	0.044
	Shoot Wash	2	0.107	0.006	0.131	0.070
	Shoot Wash	3	0.078	0.025	0.071	0.010
	Shoot Wash	4	0.064	0.004	0.092	0.032
	Shoot Wash	5	0.106	0.040	0.064	0.004
	Shoot	1	1.520	N/A	0.808	N/A
	Shoot	2	0.915	N/A	0.638	N/A
	Shoot	3	2.849	3.314	2.447	2.660
	Shoot	4	1.033	0.321	8.613	12.558
	Shoot	5	1.464	0.402	7.001	8.097
	Root	1	0.183	N/A	0.000	N/A
	Root	2	0	N/A	0.000	N/A
	Root	3	0.000	N/A	0.000	N/A
	Root	4	0.000	N/A	0.000	N/A
	Root	5	3.585	N/A	0.000	N/A
	Soil - Top Layer	1	26.421	6.049	23.012	2.924
	Soil - Top Layer	2	36.264	9.520	24.077	3.242
	Soil - Top Layer	3	32.581	7.102	28.958	10.104
	Soil - Top Layer	4	21.356	0.480	22.138	2.488
	Soil - Top Layer	5	28.259	6.004	28.709	15.047
	Soil - Middle Layer	1	27.692	6.537	22.314	4.377
	Soil - Middle Layer	2	25.000	9.348	27.214	6.856
	Soil - Middle Layer	3	24.707	9.413	36.273	19.607
	Soil - Middle Layer	4	62.929	45.518	50.161	47.301
	Soil - Middle Layer	5	22.920	1.918	28.941	4.851
	Soil - Bottom Layer	1	22.498	3.329	27.358	7.278
	Soil - Bottom Layer	2	22.684	1.751	26.140	8.510
	Soil - Bottom Layer	3	25.014	8.333	25.275	3.220
	Soil - Bottom Layer	4	20.464	1.922	32.615	3.002
	Soil - Bottom Layer	5	32.368	15.804	23.846	5.586
Monensin Sodium	Shoot Wash	1	1.100	0.147	0.041	0.016
	Shoot Wash	2	1.224	0.165	0.016	0.020
	Shoot Wash	3	1.807	0.195	0.116	N/A
	Shoot Wash	4	1.460	0.328	0.022	0.016
	Shoot Wash	5	1.169	0.916	0.062	0.028
	Shoot	1	22.161	N/A	19.317	N/A
	Shoot	2	23.451	N/A	0.029	N/A
	Shoot	3	18.565	7.335	5.679	4.597
	Shoot	4	25.106	12.817	1.134	0.210
	Shoot	5	26.417	3.529	3.180	N/A
	Root	1	5.120	N/A	9.634	N/A
	Root	2	3.479	N/A	9.345	N/A
	Root	3	7.698	N/A	4.164	N/A
	Root	4	23.758	N/A	0.126	N/A
	Root	5	2.331	N/A	1.614	N/A
	Soil - Top Layer	1	3.134	1.427	15.216	4.560
	Soil - Top Layer	2	0.000	0.000	1.328	1.875
	Soil - Top Layer	3	0.000	0.000	11.832	12.294
	Soil - Top Layer	4	13.499	14.523	5.659	6.875
	Soil - Top Layer	5	1.889	2.488	5.729	2.801
	Soil - Middle Layer	1	31.314	54.237	0.434	0.439
	Soil - Middle Layer	2	0.000	0.000	5.562	9.633
	Soil - Middle Layer	3	0.401	0.694	0.000	0.000
	Soil - Middle Layer	4	15.987	25.889	7.585	12.129
	Soil - Middle Layer	5	2.942	2.765	1.539	2.666
	Soil - Bottom Layer	1	0.000	0.000	10.124	17.536
	Soil - Bottom Layer	2	0.000	0.000	3.131	5.424
	Soil - Bottom Layer	3	0.000	0.000	6.177	10.699

Table 8. (cont'd)

	Soil - Bottom Layer	4	0.350	0.606	0.000	0.000
	Soil - Bottom Layer	5	3.550	3.990	3.547	6.144
Tylosin	Shoot Wash	1	0.334	0.060	N/A	N/A
	Shoot Wash	2	0.233	0.174	N/A	N/A
	Shoot Wash	3	1.231	0.048	0.030	0.006
	Shoot Wash	4	0.611	0.262	0.012	N/A
	Shoot Wash	5	0.401	0.165	0.095	N/A
	Shoot	1	9.946	N/A	6.167	N/A
	Shoot	2	12.141	N/A	0.000	N/A
	Shoot	3	21.045	6.375	0.068	0.117
	Shoot	4	14.337	8.577	0.174	0.124
	Shoot	5	16.070	4.436	0.337	0.298
	Root	1	5.340	N/A	2.076	N/A
	Root	2	3.868	N/A	1.183	N/A
	Root	3	5.627	N/A	0.523	N/A
	Root	4	14.596	N/A	3.697	N/A
	Root	5	7.465	N/A	4.499	N/A
	Soil - Top Layer	1	0.000	0.000	6.638	2.837
	Soil - Top Layer	2	9.585	8.647	10.313	3.119
	Soil - Top Layer	3	6.792	0.255	15.704	15.171
	Soil - Top Layer	4	25.035	10.490	32.765	12.831
	Soil - Top Layer	5	34.353	20.286	47.330	1.716
	Soil - Middle Layer	1	0.000	0.000	0.000	0.000
	Soil - Middle Layer	2	0.000	0.000	0.565	0.978
	Soil - Middle Layer	3	1.521	2.634	2.959	3.428
	Soil - Middle Layer	4	1.827	3.165	0.000	0.000
	Soil - Middle Layer	5	0.000	0.000	4.566	7.909
	Soil - Bottom Layer	1	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	2	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	3	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	4	0.397	0.688	0.000	0.000
	Soil - Bottom Layer	5	0.000	0.000	0.000	0.000

Table 9. Pharmaceutical concentrations for overhead and surface irrigated plants in Trial 2 (nominal pharmaceutical concentrations of 30 µg/L in irrigation water).

Pharmaceutical	Sample Type	Harvest Week	Average Overhead Concentration (µg/kg)	Standard Deviation (µg/kg)	Average Surface Concentration (µg/kg)	Standard Deviation (µg/kg)
Acetaminophen	Shoot	1	15.219	6.000	8.964	0.276
	Shoot	2	2.321	3.120	1.724	1.431
	Shoot	3	3.878	1.617	25.062	38.892
	Shoot	4	2.322	0.339	1.229	1.132
	Shoot	5	7.345	2.216	5.386	2.804
	Root	1	141.998	12.413	138.831	29.171
	Root	2	60.514	1.429	35.778	6.878
	Root	3	37.553	25.222	33.044	20.876
	Root	4	24.987	17.303	28.122	18.506
	Root	5	20.910	12.316	11.447	7.895
	Soil - Top Layer	1	0.000	0.000	0.000	0.000
	Soil - Top Layer	2	0.000	0.000	0.000	0.000
	Soil - Top Layer	3	0.000	0.000	0.000	0.000
	Soil - Top Layer	4	0.000	0.000	0.000	0.000
	Soil - Top Layer	5	0.000	0.000	0.000	0.000
	Soil - Middle Layer	1	0.000	0.000	0.000	0.000
	Soil - Middle Layer	2	0.000	0.000	0.000	0.000
	Soil - Middle Layer	3	0.000	0.000	0.000	0.000
	Soil - Middle Layer	4	0.000	0.000	0.000	0.000
	Soil - Middle Layer	5	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	1	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	2	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	3	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	4	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	5	0.000	0.000	0.000	0.000
Caffeine	Shoot	1	12.096	7.090	1.368	0.760
	Shoot	2	5.217	0.212	1.505	0.761
	Shoot	3	3.522	1.445	4.029	0.979
	Shoot	4	5.103	1.859	0.492	0.112
	Shoot	5	11.577	6.222	2.921	1.503
	Root	1	2.477	3.945	0.289	0.403
	Root	2	2.293	2.782	1.633	0.134
	Root	3	1.921	2.011	1.167	0.809
	Root	4	0.224	0.388	0.132	0.229
	Root	5	0.761	0.750	1.409	2.149
	Soil - Top Layer	1	7.300	2.916	0.323	0.560
	Soil - Top Layer	2	13.342	11.586	93.699	146.385
	Soil - Top Layer	3	24.594	11.511	14.391	3.824
	Soil - Top Layer	4	32.063	29.446	17.150	5.098
	Soil - Top Layer	5	32.313	1.715	11.036	2.528
	Soil - Middle Layer	1	0.000	0.000	2.357	4.082
	Soil - Middle Layer	2	0.000	0.000	6.862	8.230
	Soil - Middle Layer	3	0.000	0.000	11.239	10.743
	Soil - Middle Layer	4	0.000	0.000	0.000	0.000
	Soil - Middle Layer	5	3.311	5.735	0.000	0.000
	Soil - Bottom Layer	1	26.369	45.673	2.748	4.760
	Soil - Bottom Layer	2	1.888	3.271	33.028	57.206
	Soil - Bottom Layer	3	0.000	0.000	2.800	2.492
	Soil - Bottom Layer	4	14.756	25.558	0.000	0.000
	Soil - Bottom Layer	5	0.000	0.000	0.000	0.000
Carbamazepine	Shoot	1	41.137	3.122	10.776	2.807
	Shoot	2	86.377	7.478	52.265	21.247
	Shoot	3	94.579	10.591	129.768	19.440
	Shoot	4	166.533	32.160	224.616	38.441

Table 9. (cont'd)

	Shoot	5	326.507	99.080	344.673	139.161
	Root	1	5.341	2.386	2.082	0.374
	Root	2	7.220	2.334	10.393	4.636
	Root	3	10.683	3.198	14.505	2.214
	Root	4	15.667	3.708	21.203	2.433
	Root	5	36.961	5.758	31.289	3.062
	Soil - Top Layer	1	17.659	5.688	12.137	3.537
	Soil - Top Layer	2	24.434	4.004	26.869	3.306
	Soil - Top Layer	3	39.180	5.601	42.427	7.508
	Soil - Top Layer	4	59.613	21.045	61.619	4.898
	Soil - Top Layer	5	74.184	1.768	56.051	14.513
	Soil - Middle Layer	1	4.016	2.266	2.958	1.952
	Soil - Middle Layer	2	9.979	4.607	20.303	27.605
	Soil - Middle Layer	3	7.603	1.236	12.285	2.203
	Soil - Middle Layer	4	17.252	5.937	12.983	6.113
	Soil - Middle Layer	5	19.634	6.414	14.952	15.010
	Soil - Bottom Layer	1	1.715	0.884	7.207	9.999
	Soil - Bottom Layer	2	1.734	0.554	2.014	1.714
	Soil - Bottom Layer	3	2.294	0.980	1.900	1.314
	Soil - Bottom Layer	4	3.521	1.559	1.691	0.868
	Soil - Bottom Layer	5	5.110	4.553	1.089	0.641
Sulfadiazine	Shoot	1	0.119	0.058	0.035	0.061
	Shoot	2	0.168	0.054	0.148	0.249
	Shoot	3	0.077	0.074	0.026	0.045
	Shoot	4	0.061	0.014	1.544	2.636
	Shoot	5	0.300	0.300	0.047	0.042
	Root	1	0.520	0.281	0.551	0.256
	Root	2	0.775	0.269	0.672	0.133
	Root	3	0.725	0.167	0.900	0.169
	Root	4	0.699	0.244	0.801	0.034
	Root	5	1.242	0.296	1.050	0.241
	Soil - Top Layer	1	2.065	0.697	0.864	0.350
	Soil - Top Layer	2	1.588	0.204	1.589	0.199
	Soil - Top Layer	3	2.738	0.300	1.651	0.301
	Soil - Top Layer	4	2.575	0.544	2.500	0.371
	Soil - Top Layer	5	3.216	0.113	2.348	0.781
	Soil - Middle Layer	1	0.392	0.369	1.576	2.253
	Soil - Middle Layer	2	0.694	0.204	0.556	0.388
	Soil - Middle Layer	3	0.581	0.169	0.674	0.233
	Soil - Middle Layer	4	0.761	0.104	0.764	0.173
	Soil - Middle Layer	5	0.850	0.088	0.595	0.539
	Soil - Bottom Layer	1	0.381	0.154	0.237	0.252
	Soil - Bottom Layer	2	0.184	0.142	0.269	0.243
	Soil - Bottom Layer	3	0.252	0.063	0.175	0.153
	Soil - Bottom Layer	4	0.858	1.049	0.252	0.116
	Soil - Bottom Layer	5	0.464	0.198	0.093	0.081
Sulfamethoxazole	Shoot	1	0.000	0.000	0.153	0.264
	Shoot	2	0.000	0.000	0.000	0.000
	Shoot	3	0.035	0.060	0.008	0.007
	Shoot	4	0.019	0.033	0.000	0.000
	Shoot	5	0.572	0.072	0.061	0.105
	Root	1	1.272	1.228	0.607	0.091
	Root	2	0.932	0.206	0.743	0.167
	Root	3	4.684	5.518	1.294	0.199
	Root	4	2.320	1.793	2.121	1.355
	Root	5	1.790	0.413	1.728	0.522
	Soil - Top Layer	1	0.000	0.000	0.000	0.000
	Soil - Top Layer	2	0.000	0.000	0.000	0.000
	Soil - Top Layer	3	41.968	72.691	2.801	4.851
	Soil - Top Layer	4	0.000	0.000	2.158	3.738
	Soil - Top Layer	5	2.387	4.135	6.781	11.746

Table 9. (cont'd)

	Soil - Middle Layer	1	0.000	0.000	0.000	0.000
	Soil - Middle Layer	2	0.027	0.047	0.000	0.000
	Soil - Middle Layer	3	0.463	0.114	0.250	0.254
	Soil - Middle Layer	4	0.620	0.442	0.555	0.119
	Soil - Middle Layer	5	1.156	0.529	0.947	0.537
	Soil - Bottom Layer	1	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	2	0.000	0.000	9.343	16.183
	Soil - Bottom Layer	3	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	4	0.088	0.153	0.000	0.000
	Soil - Bottom Layer	5	0.231	0.400	0.000	0.000
Carbadox	Shoot	1	0.874	0.208	0.718	0.386
	Shoot	2	1.334	0.208	0.576	0.275
	Shoot	3	1.096	0.379	1.371	0.153
	Shoot	4	2.346	0.980	2.381	0.716
	Shoot	5	2.235	0.444	3.310	1.241
	Root	1	1.803	0.863	0.468	0.434
	Root	2	3.086	1.089	2.093	1.209
	Root	3	5.053	1.852	3.028	1.048
	Root	4	5.189	1.036	6.269	0.935
	Root	5	7.100	3.738	7.940	2.141
	Soil - Top Layer	1	1.198	0.207	0.403	0.217
	Soil - Top Layer	2	2.330	3.107	0.536	0.430
	Soil - Top Layer	3	9.169	6.694	10.262	4.167
	Soil - Top Layer	4	18.541	17.229	14.414	2.767
	Soil - Top Layer	5	10.173	8.069	9.700	6.739
	Soil - Middle Layer	1	0.392	0.080	0.187	0.138
	Soil - Middle Layer	2	0.034	0.059	1.371	1.219
	Soil - Middle Layer	3	0.111	0.107	1.961	1.773
	Soil - Middle Layer	4	0.579	0.513	1.063	1.301
	Soil - Middle Layer	5	0.790	0.697	2.330	3.737
	Soil - Bottom Layer	1	1.273	1.762	0.247	0.221
	Soil - Bottom Layer	2	0.960	0.858	1.495	2.392
	Soil - Bottom Layer	3	0.211	0.224	1.490	2.207
	Soil - Bottom Layer	4	0.379	0.336	0.035	0.060
	Soil - Bottom Layer	5	0.024	0.042	0.919	0.754
Trimethoprim	Shoot	1	0.789	0.145	0.049	0.057
	Shoot	2	0.420	0.221	0.010	0.005
	Shoot	3	0.155	0.056	0.021	0.008
	Shoot	4	0.168	0.126	0.010	0.001
	Shoot	5	0.128	0.026	0.025	0.013
	Root	1	1.276	0.953	0.320	0.338
	Root	2	2.220	0.987	2.403	1.929
	Root	3	3.163	0.309	6.283	1.124
	Root	4	5.505	2.300	4.437	1.449
	Root	5	8.589	3.145	7.467	2.142
	Soil - Top Layer	1	0.000	0.000	0.000	0.000
	Soil - Top Layer	2	0.000	0.000	0.000	0.000
	Soil - Top Layer	3	0.253	0.437	3.297	5.711
	Soil - Top Layer	4	31.990	36.166	8.791	1.825
	Soil - Top Layer	5	24.791	3.969	4.670	4.088
	Soil - Middle Layer	1	0.000	0.000	0.000	0.000
	Soil - Middle Layer	2	0.000	0.000	0.000	0.000
	Soil - Middle Layer	3	0.000	0.000	0.000	0.000
	Soil - Middle Layer	4	0.000	0.000	0.000	0.000
	Soil - Middle Layer	5	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	1	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	2	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	3	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	4	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	5	0.000	0.000	0.000	0.000
Lincomycin	Shoot	1	2.311	0.347	0.866	0.209

Table 9. (cont'd)

	Shoot	2	3.773	0.887	0.929	0.273
	Shoot	3	3.031	0.312	1.668	0.489
	Shoot	4	5.584	1.897	3.169	0.418
	Shoot	5	5.800	1.439	3.867	1.138
	Root	1	1.621	0.441	0.438	0.075
	Root	2	3.009	0.207	2.613	1.147
	Root	3	4.057	0.423	3.062	0.802
	Root	4	4.158	0.980	3.267	0.097
	Root	5	5.699	2.312	3.840	0.833
	Soil - Top Layer	1	2.663	1.273	65.561	112.342
	Soil - Top Layer	2	4.937	0.081	6.488	0.697
	Soil - Top Layer	3	117.727	188.556	9.673	2.763
	Soil - Top Layer	4	14.053	7.741	12.674	1.395
	Soil - Top Layer	5	7.887	6.923	10.832	2.133
	Soil - Middle Layer	1	0.000	0.000	0.000	0.000
	Soil - Middle Layer	2	0.157	0.273	0.000	0.000
	Soil - Middle Layer	3	0.000	0.000	2.775	4.807
	Soil - Middle Layer	4	2.081	1.961	1.301	1.175
	Soil - Middle Layer	5	0.786	1.361	1.547	2.680
	Soil - Bottom Layer	1	11.888	20.590	0.000	0.000
	Soil - Bottom Layer	2	0.000	0.000	12.720	22.032
	Soil - Bottom Layer	3	0.000	0.000	2.443	4.231
	Soil - Bottom Layer	4	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	5	0.000	0.000	1.869	3.237
Oxytetracycline	Shoot	1	1.071	0.075	0.698	0.112
	Shoot	2	2.613	2.687	1.157	0.678
	Shoot	3	1.223	0.084	0.978	0.108
	Shoot	4	3.065	1.261	1.683	0.159
	Shoot	5	3.341	0.635	5.807	5.070
	Root	1	2.647	1.210	1.362	0.389
	Root	2	1.611	0.013	1.900	0.980
	Root	3	1.813	0.276	2.815	0.609
	Root	4	2.579	0.170	2.928	0.610
	Root	5	3.019	0.629	4.613	2.473
	Soil - Top Layer	1	0.000	0.000	0.000	0.000
	Soil - Top Layer	2	0.000	0.000	0.573	0.993
	Soil - Top Layer	3	0.032	0.056	0.000	0.000
	Soil - Top Layer	4	0.264	0.457	0.000	0.000
	Soil - Top Layer	5	1.397	1.720	0.000	0.000
	Soil - Middle Layer	1	0.000	0.000	0.000	0.000
	Soil - Middle Layer	2	0.000	0.000	0.000	0.000
	Soil - Middle Layer	3	0.000	0.000	0.000	0.000
	Soil - Middle Layer	4	0.000	0.000	0.000	0.000
	Soil - Middle Layer	5	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	1	0.000	0.000	0.307	0.532
	Soil - Bottom Layer	2	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	3	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	4	0.000	0.000	0.249	0.432
	Soil - Bottom Layer	5	0.000	0.000	0.000	0.000
Monensin Sodium	Shoot	1	6.327	1.397	0.000	0.000
	Shoot	2	4.593	1.601	0.000	0.000
	Shoot	3	5.020	1.394	0.000	0.000
	Shoot	4	7.081	3.904	0.000	0.000
	Shoot	5	9.137	1.453	0.731	1.153
	Root	1	0.698	0.123	0.391	0.260
	Root	2	1.416	1.511	0.710	0.444
	Root	3	0.776	0.330	1.882	1.192
	Root	4	0.228	0.198	0.552	0.325
	Root	5	1.079	0.159	1.436	1.052
	Soil - Top Layer	1	10.883	1.070	7.296	1.662

Table 9. (cont'd)

	Soil - Top Layer	2	6.844	0.820	6.428	1.198
	Soil - Top Layer	3	6.107	0.651	5.980	1.502
	Soil - Top Layer	4	5.892	3.210	4.759	0.783
	Soil - Top Layer	5	5.196	0.774	5.127	1.124
	Soil - Middle Layer	1	4.495	0.521	4.196	0.268
	Soil - Middle Layer	2	3.607	0.161	8.178	8.038
	Soil - Middle Layer	3	3.111	0.084	4.388	0.985
	Soil - Middle Layer	4	9.457	10.663	3.711	0.309
	Soil - Middle Layer	5	4.315	1.642	9.715	10.635
	Soil - Bottom Layer	1	3.938	0.457	3.325	0.219
	Soil - Bottom Layer	2	4.406	2.305	3.511	1.027
	Soil - Bottom Layer	3	2.923	0.120	4.209	2.406
	Soil - Bottom Layer	4	3.038	0.251	2.911	0.098
	Soil - Bottom Layer	5	3.696	0.696	3.556	0.620
Tylosin	Shoot	1	1.328	0.133	0.027	0.026
	Shoot	2	1.478	0.272	0.106	0.165
	Shoot	3	1.272	0.339	0.111	0.060
	Shoot	4	1.495	0.666	0.042	0.017
	Shoot	5	1.434	0.119	0.121	0.084
	Root	1	0.371	0.203	0.174	0.016
	Root	2	0.478	0.143	0.732	0.330
	Root	3	0.581	0.042	1.377	0.635
	Root	4	0.589	0.311	0.512	0.112
	Root	5	0.803	0.233	0.700	0.284
	Soil - Top Layer	1	5.789	1.215	2.003	1.179
	Soil - Top Layer	2	4.707	1.458	11.219	3.315
	Soil - Top Layer	3	14.799	3.246	17.490	4.938
	Soil - Top Layer	4	27.261	18.275	21.132	0.764
	Soil - Top Layer	5	22.323	2.774	14.209	2.447
	Soil - Middle Layer	1	0.003	0.006	0.000	0.000
	Soil - Middle Layer	2	0.913	1.582	0.000	0.000
	Soil - Middle Layer	3	0.000	0.000	0.787	1.364
	Soil - Middle Layer	4	1.340	1.407	1.505	2.608
	Soil - Middle Layer	5	0.435	0.754	3.201	5.545
	Soil - Bottom Layer	1	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	2	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	3	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	4	0.000	0.000	0.000	0.000
	Soil - Bottom Layer	5	0.146	0.254	0.000	0.000

Table 10. Root concentration factors and translocation factors for pharmaceuticals in the 30-µg/L trial.

		Overhead				Surface			
Pharmaceutical	**Harvest Week**	**Average Root Concentration Factor**	**Root Concentration Factor Standard Deviation**	**Average Translocation Factor**	**Translocation Factor Standard Deviation**	**Average Root Concentration Factor**	**Root Concentration Factor Standard Deviation**	**Average Translocation Factor**	**Translocation Factor Standard Deviation**
Acetaminophen	1	n/a	n/a	0.108	0.043	n/a	n/a	0.066	0.013
	2	n/a	n/a	0.042	0.049	n/a	n/a	0.053	0.044
	3	n/a	n/a	0.139	0.079	n/a	n/a	1.646	2.736
	4	n/a	n/a	0.183	0.196	n/a	n/a	n/a	n/a
	5	n/a	n/a	0.396	0.153	n/a	n/a	0.758	0.625
Caffeine	1	0.143	0.120	66.551	94.294	n/a	n/a	n/a	n/a
	2	0.347	0.384	22.678	35.326	0.210	0.317	0.945	0.510
	3	0.253	0.217	2.803	1.401	0.125	0.077	5.111	3.860
	4	0.010	0.017	n/a	n/a	0.034	0.058	n/a	n/a
	5	0.058	0.047	20.405	10.006	0.324	0.487	n/a	n/a
Carbamazepine	1	0.688	0.233	8.477	2.630	0.360	0.210	5.145	0.591
	2	0.623	0.287	12.651	3.260	0.677	0.140	5.077	0.272
	3	0.649	0.138	9.168	1.632	0.787	0.215	8.950	0.039
	4	0.614	0.244	11.326	4.517	0.844	0.179	10.673	2.144
	5	1.121	0.138	8.689	1.526	1.374	0.428	10.892	3.592
Sulfadiazine	1	0.274	0.216	0.274	0.216	0.129	0.224	0.129	0.224
	2	0.235	0.098	0.235	0.098	0.181	0.302	0.181	0.302
	3	0.108	0.118	0.108	0.118	0.033	0.056	0.033	0.056
	4	0.092	0.024	0.092	0.024	1.972	3.371	1.972	3.371
	5	0.213	0.192	0.213	0.192	0.045	0.040	0.045	0.040
Sulfamethoxazole	1	n/a	n/a	n/a	n/a	n/a	n/a	0.298	0.516
	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	3	33.438	40.156	0.017	0.030	n/a	n/a	0.006	0.005
	4	21.728	34.273	0.011	0.019	13.089	11.427	n/a	n/a
	5	2.359	1.763	0.325	0.036	6.646	3.661	0.026	0.045
Carbadox	1	2.472	1.787	0.584	0.332	1.753	1.589	n/a	n/a
	2	3.538	1.976	0.464	0.149	5.584	7.313	0.310	0.175
	3	2.490	1.950	0.221	0.034	0.648	0.107	0.488	0.155
	4	1.086	0.518	0.439	0.099	1.281	0.464	0.398	0.188
	5	2.957	2.376	0.412	0.277	3.008	2.192	0.465	0.268
Trimethoprim	1	n/a	n/a	0.840	0.463	n/a	n/a	0.058	0.064
	2	n/a	n/a	0.213	0.110	n/a	n/a	0.002	0.002
	3	n/a	n/a	0.048	0.013	n/a	n/a	0.003	0.001
	4	16.444	27.824	0.040	0.041	1.533	0.524	0.003	0.001
	5	1.031	0.272	0.016	0.007	n/a	n/a	0.004	0.003
Lincomycin	1	1.354	1.377	1.479	0.382	1.496	1.458	1.980	0.292
	2	1.778	0.181	1.253	0.288	0.885	0.820	0.383	0.104
	3	0.893	0.760	0.748	0.052	0.625	0.184	0.552	0.113
	4	0.891	0.543	1.340	0.382	0.718	0.145	0.968	0.103
	5	n/a	n/a	1.074	0.254	0.910	0.340	1.000	0.188
Oxytetracycline	1	n/a	n/a	0.464	0.196	n/a	n/a	0.540	0.166

Table 10. (cont'd)

	2	n/a	n/a	1.629	1.684	n/a	n/a	0.777	0.657
	3	n/a	n/a	0.681	0.066	n/a	n/a	0.354	0.053
	4	n/a	n/a	1.193	0.490	n/a	n/a	0.597	0.162
	5	n/a	n/a	1.136	0.275	n/a	n/a	1.236	0.597
Monensin Sodium	1	0.108	0.012	9.297	2.669	0.081	0.057	n/a	n/a
	2	0.258	0.239	6.942	5.218	0.118	0.059	n/a	n/a
	3	0.193	0.086	7.573	3.922	0.371	0.157	n/a	n/a
	4	0.039	0.043	n/a	n/a	0.144	0.086	n/a	n/a
	5	0.251	0.060	8.463	0.370	0.266	0.237	0.561	0.928
Tylosin	1	0.184	0.070	4.776	3.526	0.360	0.264	0.165	0.169
	2	0.260	0.098	3.359	1.343	0.189	0.035	0.107	0.150
	3	0.121	0.021	2.181	0.486	0.235	0.128	0.118	0.125
	4	0.086	0.077	3.158	2.404	0.068	0.011	0.086	0.037
	5	0.107	0.038	1.878	0.493	0.121	0.024	0.201	0.183

Table 11. Mass balance for pharmaceuticals in the 50-µg/L trial.

			Overhead				Surface			
Pharmaceutical	Harvest Week	Cumulative amount of Pharmaceutical Applied in Irrigation Water (µg)	Total Shoot Accumulation (µg)	Total Root Accumulation (µg)	Total Soil Accumulation (µg)	% Captured	Total Shoot Accumulation (µg)	Total Root Accumulation (µg)	Total Soil Accumulation (µg)	% Captured
Acetaminophen	1	26.936	0.289	0.002	3.438	13.8	0.262	0.000	2.065	8.6
	2	56.647	0.116	0.123	2.295	4.5	0.246	0.014	3.110	5.9
	3	87.850	0.233	0.115	3.943	4.9	0.185	0.101	2.780	3.5
	4	121.434	0.355	0.146	5.371	4.8	0.211	0.061	2.585	2.4
	5	161.468	0.588	0.141	3.655	2.7	0.313	0.000	3.678	2.5
Caffeine	1	22.615	0.303	0.709	22.063	102.0	0.135	0.386	48.143	215.2
	2	50.203	0.929	0.070	38.805	79.3	0.065	0.190	30.671	61.6
	3	80.385	0.870	0.250	58.625	74.3	1.243	0.529	43.256	56.0
	4	112.182	1.228	0.662	46.802	43.4	0.862	0.188	46.487	42.4
	5	147.840	1.394	0.058	28.999	20.6	0.878	1.222	67.364	47.0
Carbamazepine	1	27.050	0.207	1.134	9.887	41.5	0.067	0.044	51.113	189.4
	2	57.066	1.782	0.355	36.282	67.3	0.236	0.019	24.964	44.2
	3	88.138	1.949	0.200	39.089	46.8	1.684	0.030	41.288	48.8
	4	122.485	1.652	0.627	58.485	49.6	2.130	0.190	65.246	55.2
	5	163.449	2.940	0.071	83.263	52.8	2.590	0.090	87.449	55.1
Sulfadiazine	1	24.860	0.000	0.000	2.727	11.0	0.000	0.007	1.446	5.8
	2	53.530	0.000	0.001	0.911	1.7	0.000	0.000	0.190	0.4
	3	83.307	0.001	0.004	0.842	1.0	0.003	0.002	2.726	3.3
	4	115.445	0.008	0.006	3.303	2.9	0.000	0.003	6.939	6.0
	5	151.642	0.000	0.002	0.813	0.5	0.023	0.003	1.886	1.3
Sulfamethoxazole	1	26.654	0.100	0.064	5.467	21.1	0.008	0.099	0.000	0.4
	2	55.812	0.012	0.030	0.000	0.1	0.000	0.012	36.130	64.8
	3	87.825	1.810	0.016	27.282	33.1	1.042	0.015	6.332	8.4
	4	121.498	0.060	0.136	22.848	19.0	0.022	0.006	9.245	7.6
	5	161.967	0.366	0.010	35.734	22.3	0.000	0.029	19.395	12.0
Carbadox	1	21.250	0.033	0.044	3.200	15.4	0.018	0.165	1.402	7.5
	2	47.500	0.016	0.009	1.282	2.8	0.000	0.009	0.000	0.0
	3	71.250	0.031	0.015	0.000	0.1	0.155	0.023	5.709	8.3
	4	97.500	0.000	0.019	42.999	44.1	0.000	0.000	11.456	11.8
	5	128.750	0.000	0.056	2.796	2.2	0.000	0.026	4.446	3.5
Trimethoprim	1	25.661	0.255	0.017	2.885	12.3	0.003	0.049	7.047	27.7
	2	57.036	0.217	0.047	9.572	17.2	0.006	0.013	12.391	21.8
	3	90.674	0.816	0.061	10.057	12.1	0.115	0.023	24.416	27.1
	4	126.924	0.604	0.090	20.214	16.5	0.033	0.025	48.845	38.5
	5	168.703	0.571	0.080	57.033	34.2	0.022	0.047	54.571	32.4
Lincomycin	1	23.305	0.072	0.045	16.183	69.9	0.144	1.772	40.573	182.3
	2	50.148	0.229	1.330	26.536	56.0	0.028	2.067	26.950	57.9
	3	77.402	2.415	0.058	11.987	18.7	3.314	0.451	17.895	28.0

Table 11. (cont'd)

	4	107.921	0.358	0.673	36.067	34.4	0.365	0.072	91.934	85.6
	5	143.343	0.250	0.051	6.252	4.6	0.086	0.046	12.500	8.8
Oxytetracycline	1	21.250	0.004	0.000	36.184	170.3	0.002	0.000	27.032	127.2
	2	47.500	0.006	0.000	34.028	71.6	0.004	0.000	36.571	77.0
	3	71.250	0.040	0.000	38.872	54.6	0.027	0.000	42.747	60.0
	4	97.500	0.020	0.000	49.474	50.8	0.135	0.000	49.551	51.0
	5	128.750	0.030	0.012	39.460	30.7	0.122	0.000	38.491	30.0
Monensin Sodium	1	19.819	0.064	0.005	16.270	82.4	0.046	0.049	12.173	61.9
	2	38.931	0.152	0.009	0.000	0.4	0.000	0.018	4.733	12.2
	3	59.871	0.253	0.028	0.189	0.8	0.059	0.017	8.506	14.3
	4	79.014	0.479	0.088	13.926	18.3	0.011	0.001	8.640	10.9
	5	105.547	0.514	0.008	3.958	4.2	0.013	0.005	5.108	4.9
Tylosin	1	23.653	0.029	0.006	0.000	0.1	0.015	0.003	3.135	13.3
	2	51.255	0.079	0.010	4.527	9.0	0.000	0.002	5.137	10.0
	3	80.510	0.288	0.021	3.926	5.3	0.002	0.002	8.815	11.0
	4	112.134	0.274	0.054	12.875	11.8	0.003	0.017	15.475	13.8
	5	148.649	0.307	0.026	16.225	11.1	0.008	0.015	24.511	16.5

Table 12. Mass balance for pharmaceuticals in the 30-µg/L trial.

			Overhead				Surface			
Pharmaceutical	**Harvest Week**	**Cumulative amount of Pharmaceutical Applied in Irrigation Water (µg)**	**Total Shoot Accumulation (µg)**	**Total Root Accumulation (µg)**	**Total Soil Accumulation (µg)**	**% Captured**	**Total Shoot Accumulation (µg)**	**Total Root Accumulation (µg)**	**Total Soil Accumulation (µg)**	**% Captured**
Acetaminophen	1	9.076	0.298	0.918	0.000	13.4	0.214	0.838	0.000	11.6
	2	22.965	0.090	0.571	0.000	2.9	0.106	0.278	0.000	1.7
	3	49.541	0.294	0.476	0.000	1.6	2.071	0.345	0.000	4.9
	4	81.136	0.191	0.200	0.000	0.5	0.093	0.146	0.000	0.3
	5	113.991	0.658	0.211	0.000	0.8	0.371	0.109	0.000	0.4
Caffeine	1	14.093	0.243	0.018	15.902	114.7	0.032	0.001	2.564	18.4
	2	36.929	0.208	0.021	7.193	20.1	0.093	0.013	63.095	171.1
	3	66.343	0.267	0.023	11.616	17.9	0.327	0.012	13.428	20.8
	4	100.660	0.405	0.006	22.113	22.4	0.037	0.002	8.100	8.1
	5	137.041	1.039	0.007	16.826	13.0	0.204	0.016	5.212	4.0
Carbamazepine	1	16.408	0.822	0.036	11.047	72.6	0.252	0.012	10.533	65.8
	2	41.413	3.441	0.066	17.073	49.7	3.219	0.081	23.231	64.1
	3	73.383	7.121	0.128	23.179	41.5	10.487	0.151	26.738	50.9
	4	109.501	13.607	0.136	37.967	47.2	16.714	0.170	36.034	48.3
	5	148.076	29.290	0.387	46.724	51.6	23.339	0.289	34.049	39.0
Sulfadiazine	1	15.941	0.002	0.003	1.341	8.4	0.001	0.003	1.264	8.0
	2	38.187	0.007	0.008	1.165	3.1	0.009	0.005	1.140	3.0
	3	66.831	0.006	0.009	1.687	2.5	0.002	0.009	1.181	1.8
	4	99.406	0.005	0.006	1.981	2.0	0.106	0.006	4.980	5.1
	5	134.345	0.027	0.013	2.140	1.6	0.003	0.009	1.434	1.1
Sulfamethoxazole	1	7.800	0.000	0.009	19.822	254.2	0.013	0.004	0.000	0.2
	2	17.618	0.000	0.009	0.013	0.1	0.000	0.006	5.540	31.5
	3	42.586	0.008	0.049	0.219	0.6	0.001	0.013	0.118	0.3
	4	71.088	0.005	0.021	3.537	5.0	0.000	0.015	0.262	0.4
	5	102.708	0.051	0.021	1.674	1.7	0.013	0.016	0.447	0.5
Carbadox	1	15.000	0.017	0.012	1.352	9.2	0.016	0.005	0.395	2.8
	2	38.250	0.053	0.028	1.570	4.3	0.036	0.016	1.607	4.3
	3	63.000	0.083	0.062	4.483	7.3	0.111	0.032	6.476	10.5
	4	87.000	0.185	0.043	9.209	10.8	0.180	0.051	21.980	25.5
	5	113.250	0.200	0.065	5.190	4.8	0.228	0.071	6.116	5.7
Trimethoprim	1	7.800	0.016	0.009	0.000	0.3	0.001	0.002	0.000	0.0
	2	19.482	0.017	0.020	0.000	0.2	0.000	0.019	0.000	0.1
	3	47.116	0.012	0.038	0.119	0.4	0.002	0.065	1.557	3.4
	4	80.194	0.012	0.049	15.109	18.9	0.001	0.034	4.152	5.2
	5	115.562	0.011	0.087	11.709	10.2	0.002	0.067	2.205	2.0
Lincomycin	1	14.928	0.046	0.011	6.872	46.4	0.020	0.003	30.965	207.6
	2	38.814	0.151	0.029	2.406	6.7	0.058	0.020	9.072	23.6
	3	66.185	0.228	0.048	55.603	84.4	0.135	0.032	7.033	10.9

Table 12. (cont'd)

	4	97.628	0.462	0.036	7.620	8.3	0.236	0.026	6.600	7.0
	5	131.872	0.521	0.061	4.096	3.5	0.268	0.036	6.730	5.3
Oxytetracycline	1	15.000	0.021	0.017	0.000	0.3	0.017	0.008	0.145	1.1
	2	38.250	0.107	0.015	0.000	0.3	0.073	0.015	0.271	0.9
	3	63.000	0.092	0.022	0.015	0.2	0.079	0.029	0.000	0.2
	4	87.000	0.256	0.022	0.125	0.5	0.126	0.023	0.118	0.3
	5	113.250	0.300	0.031	0.660	0.9	0.503	0.039	0.000	0.5
Monensin Sodium	1	14.622	0.126	0.005	9.123	63.3	0.000	0.003	6.998	47.9
	2	36.487	0.185	0.013	7.017	19.8	0.000	0.006	8.557	23.5
	3	63.614	0.379	0.010	5.734	9.6	0.000	0.020	6.885	10.9
	4	94.879	0.546	0.003	8.685	9.7	0.000	0.004	16.127	17.0
	5	127.258	0.820	0.011	6.238	5.6	0.058	0.013	8.689	6.9
Tylosin	1	14.625	0.027	0.002	2.736	18.9	0.001	0.001	0.946	6.5
	2	36.184	0.059	0.004	2.655	7.5	0.006	0.006	5.299	14.7
	3	61.474	0.096	0.007	6.990	11.5	0.009	0.014	8.633	14.1
	4	91.157	0.117	0.005	13.509	15.0	0.003	0.004	10.692	11.7
	5	122.260	0.129	0.009	10.818	9.0	0.008	0.006	8.223	6.7

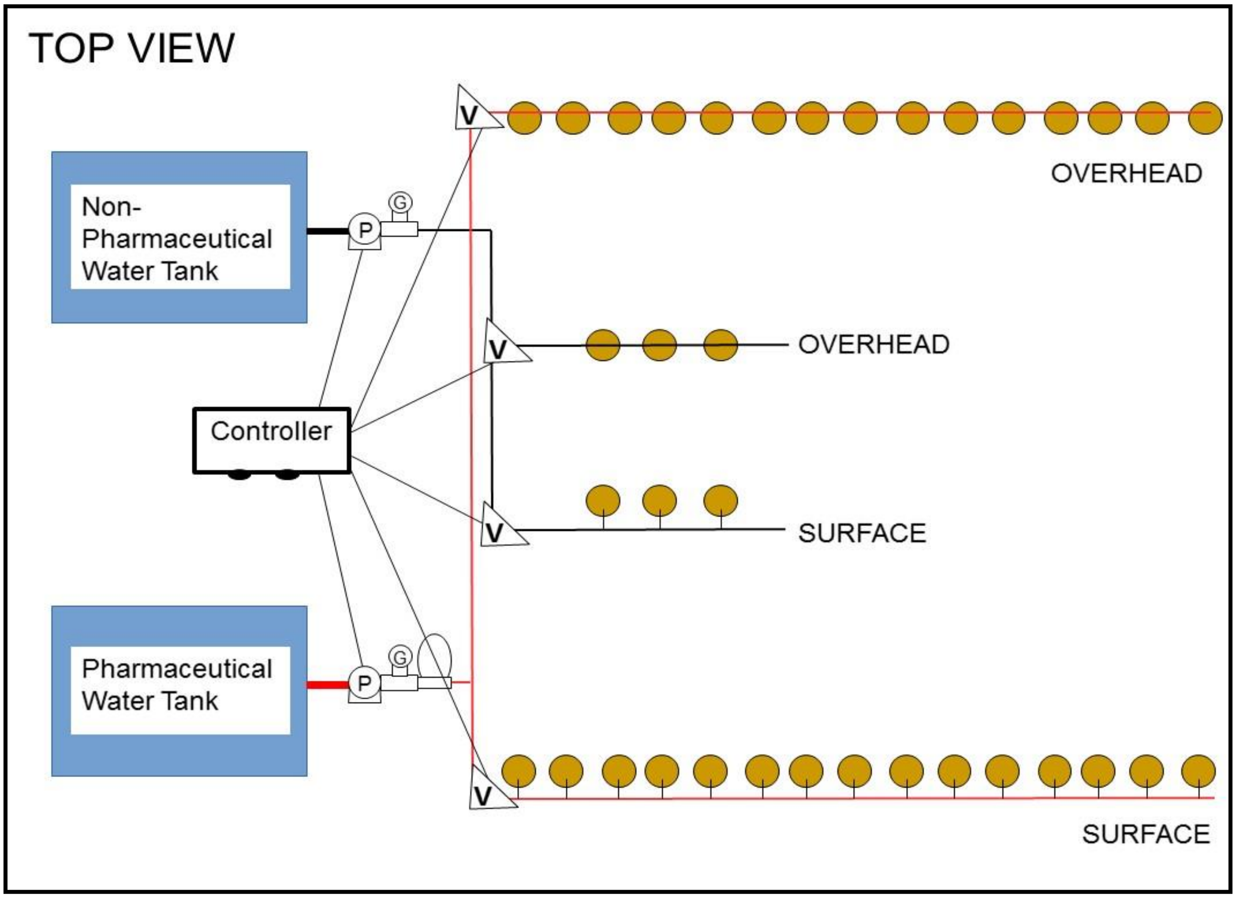


Figure 1. Schematic of automatic irrigation system (Pump = P, Pressure Gauge = G, and Valve = V).

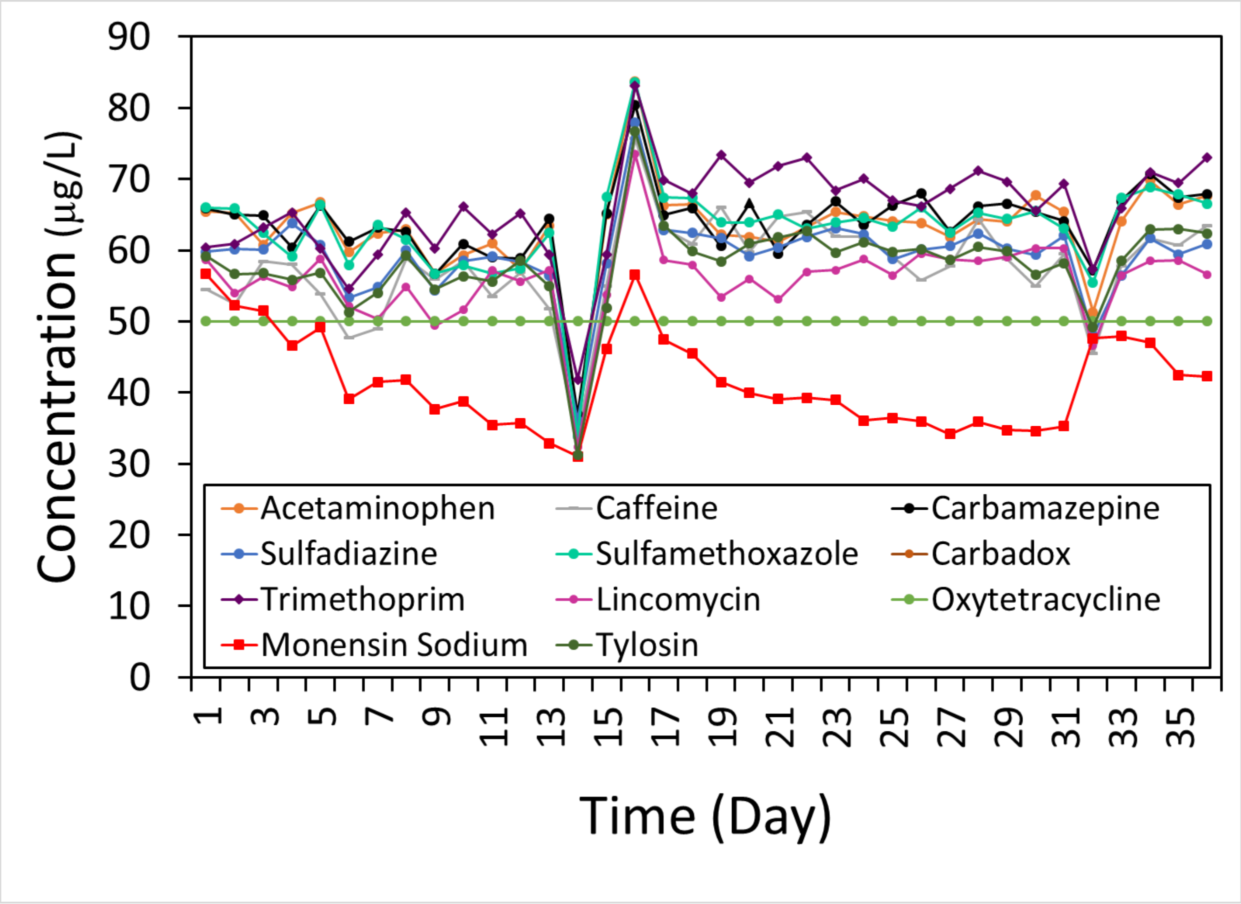


Figure 2. Pharmaceutical concentrations in irrigation water over time in 50 µg/L Trial.

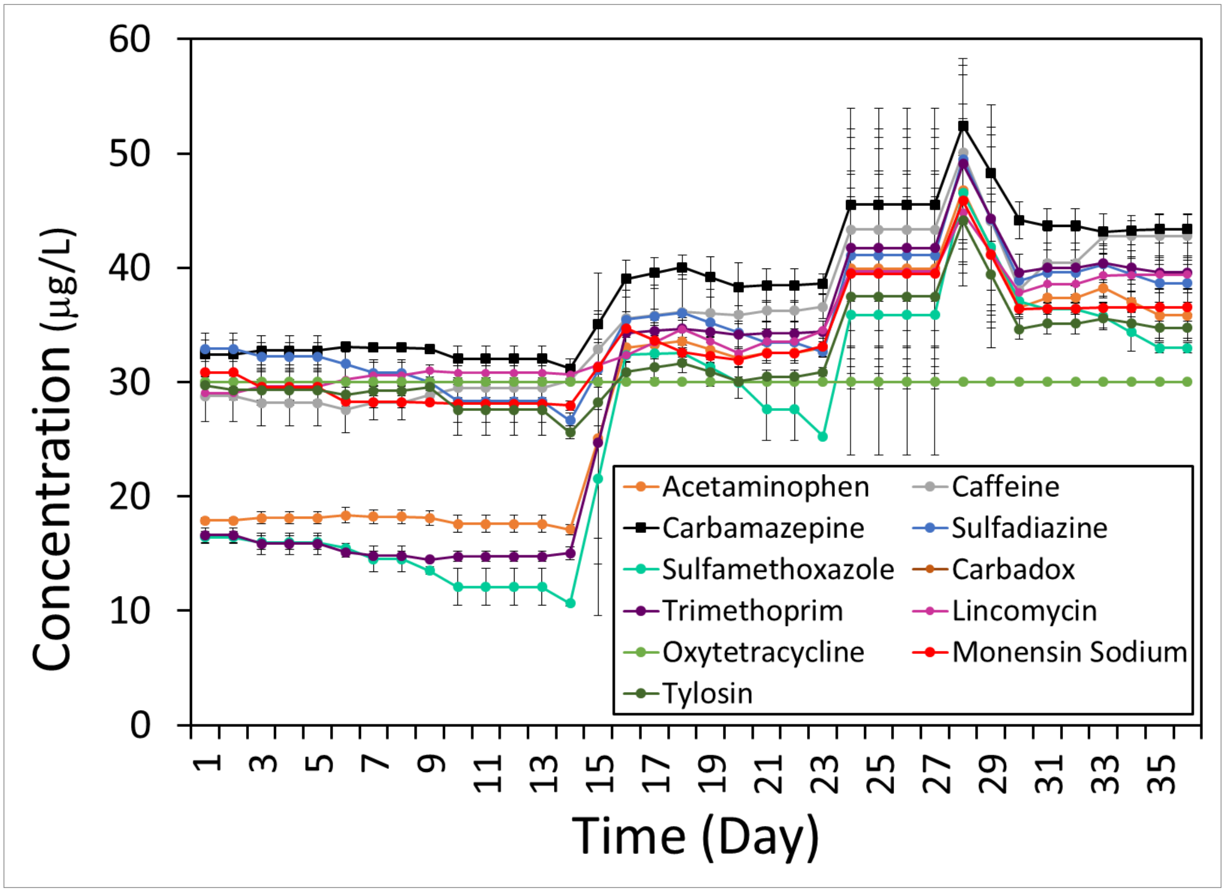


Figure 3. Pharmaceutical concentrations in irrigation water over time in 30 µg/L Trial.

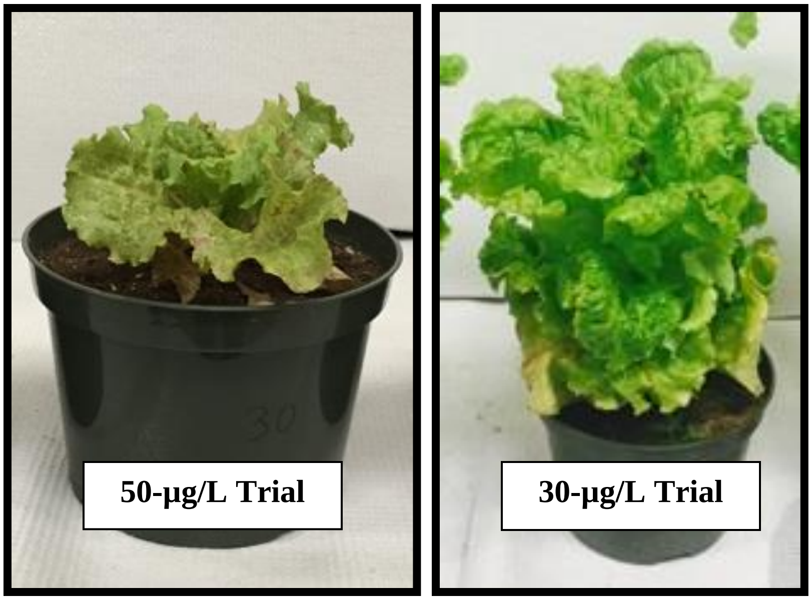


Figure 4. Images of lettuce in the 50-µg/L and 30-µg/L trials at week 3

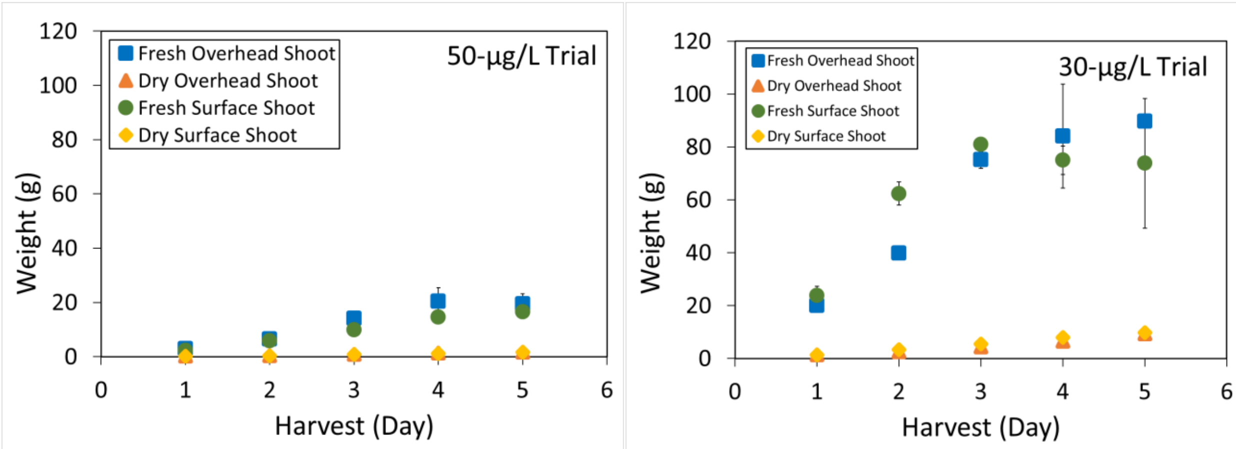


Figure 5. Fresh and dry shoot biomass for Trial 1 and Trial 2 (nominal pharmaceutical concentrations of 50 and 30 µg/L in irrigation water, respectively).

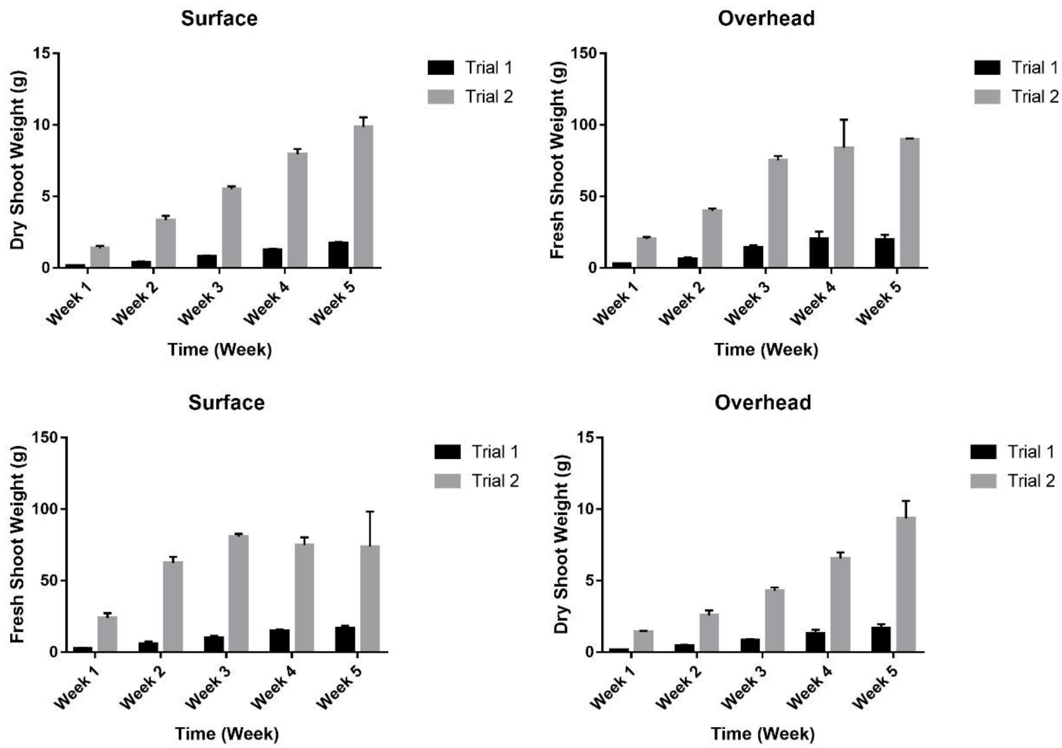


Figure 6. Holm-Sidak two-tailed unpaired t-test showing significant difference ($p < 0.05$) between plant biomass between Trial 1 and Tria 2 (Trial 1 = nominal pharmaceutical concentration of 50 µg/L in irrigation water, Trial 2 = nominal pharmaceutical concentration of 30 µg/L in irrigation water).

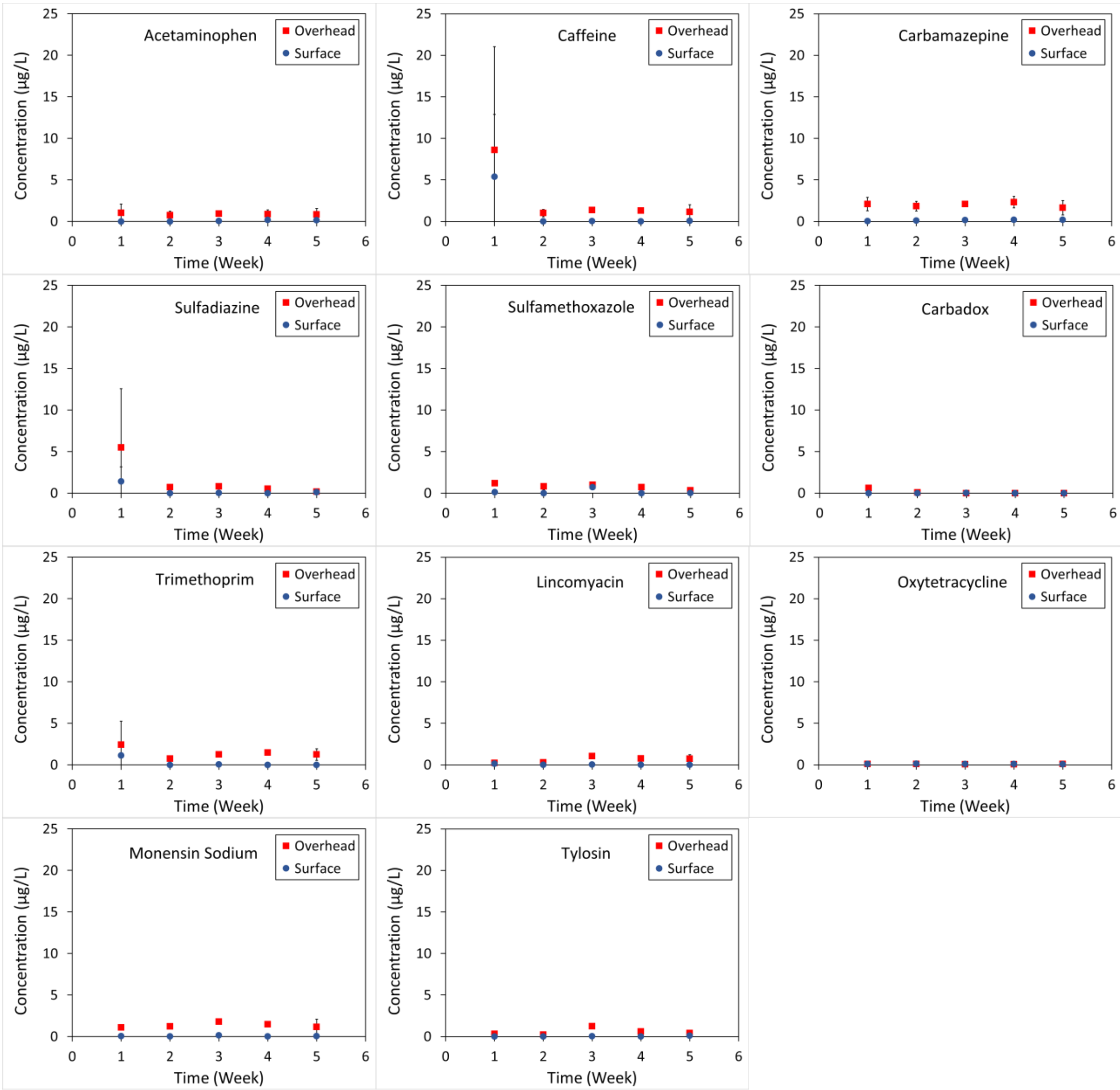


Figure 7. Pharmaceutical concentrations in shoot wash waters for overhead and surface irrigated plants in Trial 1 (nominal pharmaceutical concentrations of 50 µg/L in irrigation water).

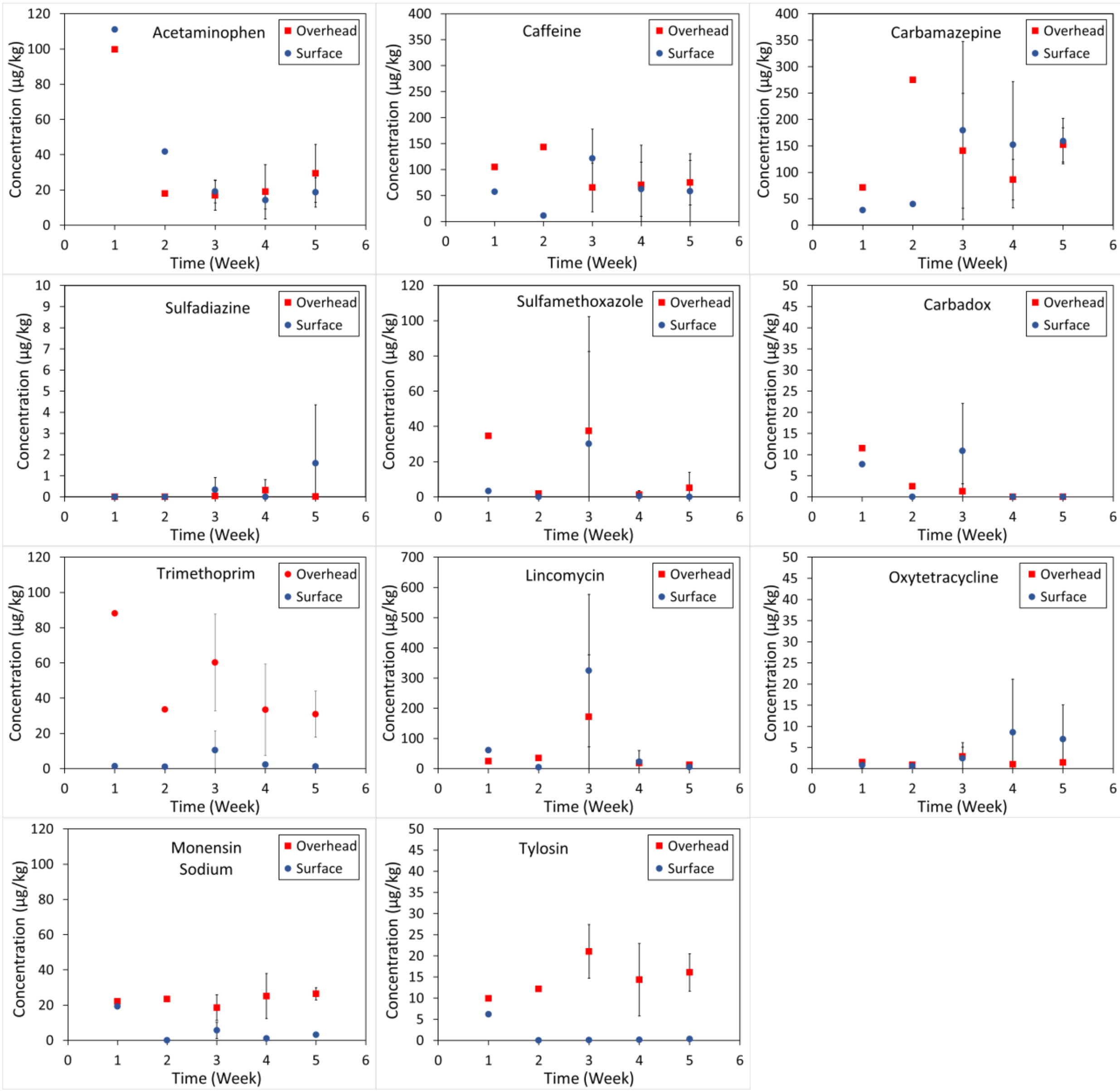


Figure 8. Pharmaceutical concentrations in lettuce shoots for overhead and surface irrigated plants in Trial 1 (nominal pharmaceutical concentrations of 50 µg/L in irrigation water).

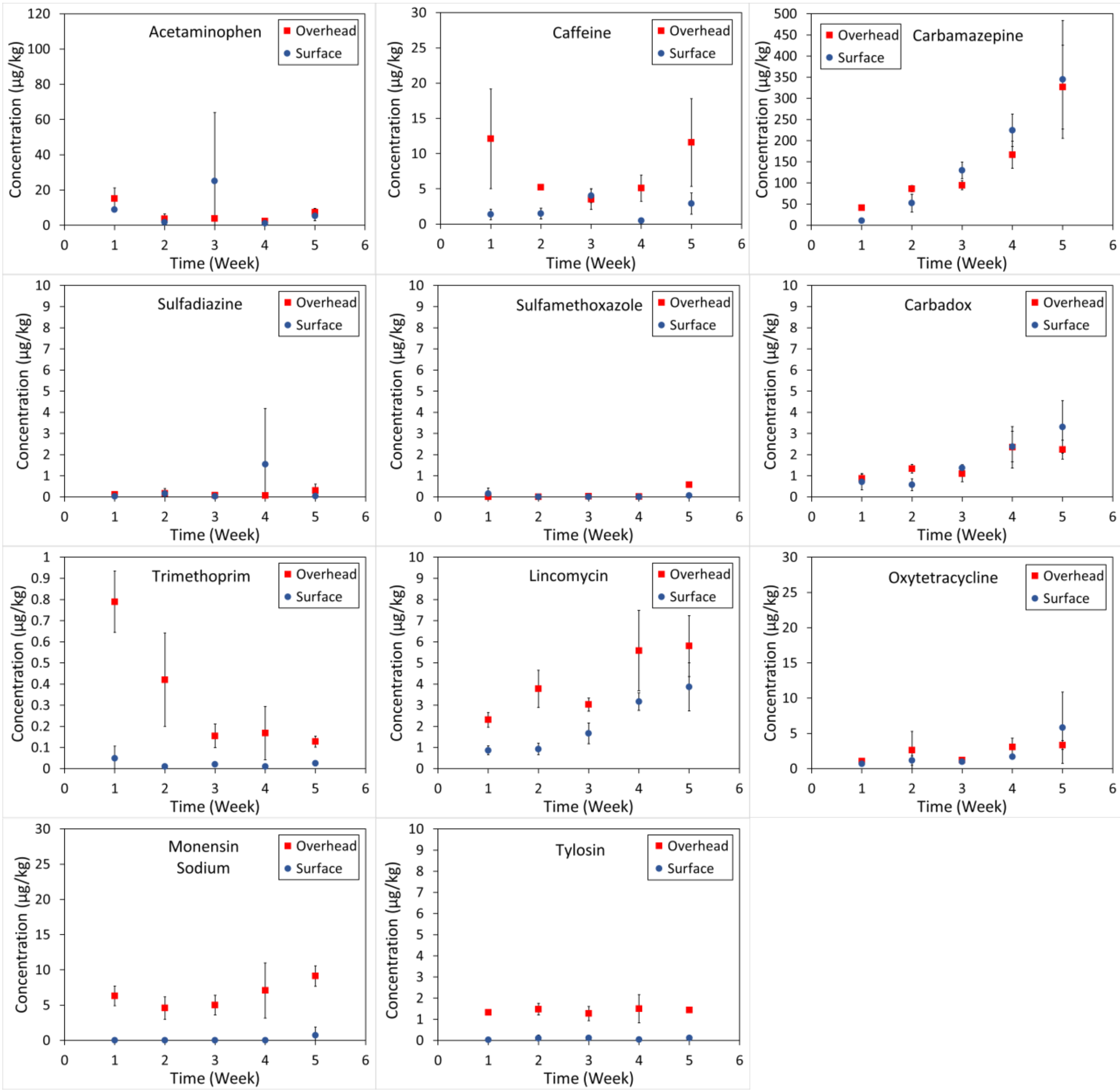


Figure 9. Pharmaceutical concentrations in lettuce shoots for overhead and surface irrigated plants in Trial 2 (nominal pharmaceutical concentrations of 30 µg/L in irrigation water).

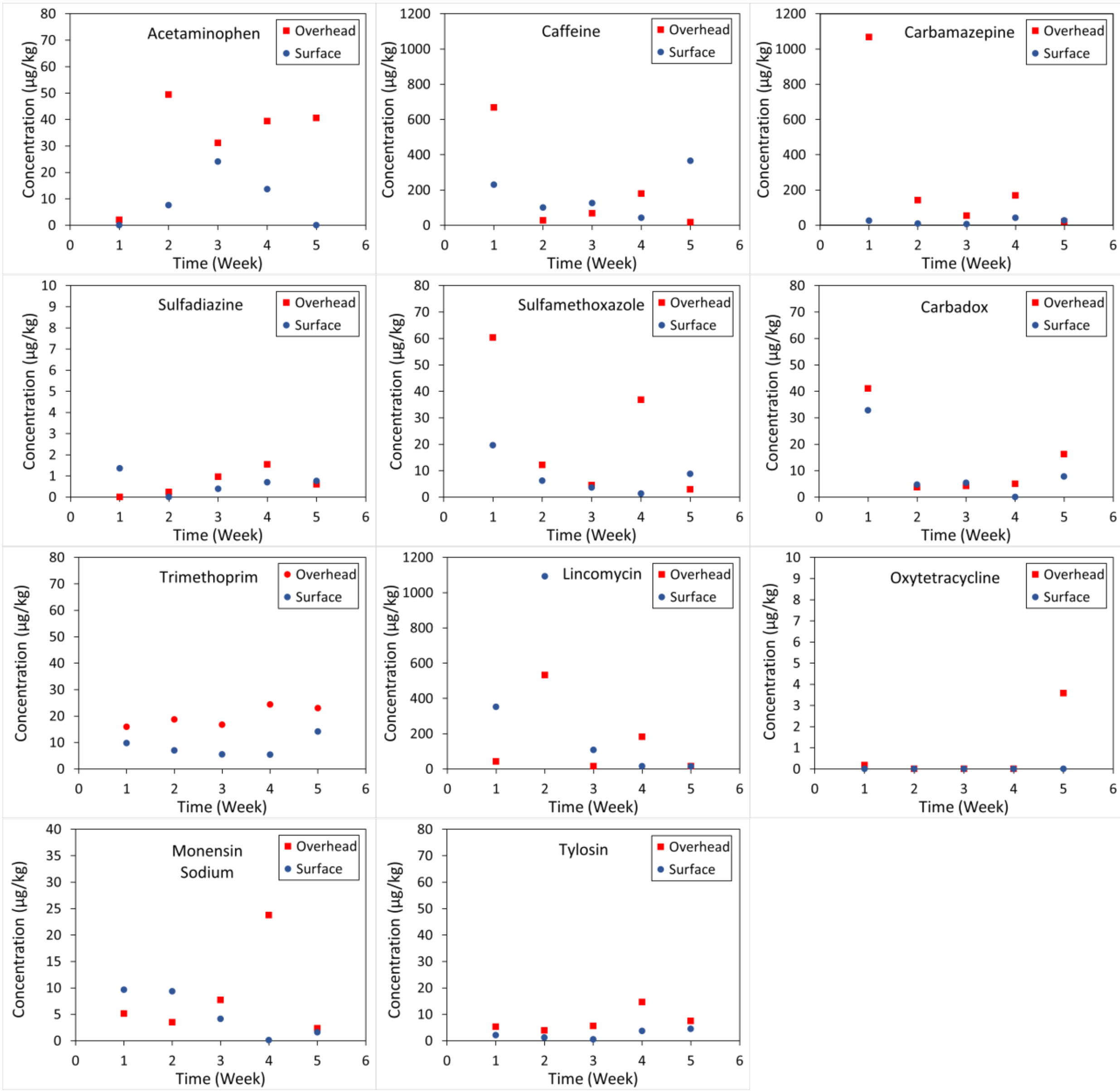


Figure 10. Pharmaceutical concentrations in lettuce roots for overhead and surface irrigated plants in Trial 1 (nominal pharmaceutical concentrations of 50 µg/L in irrigation water).

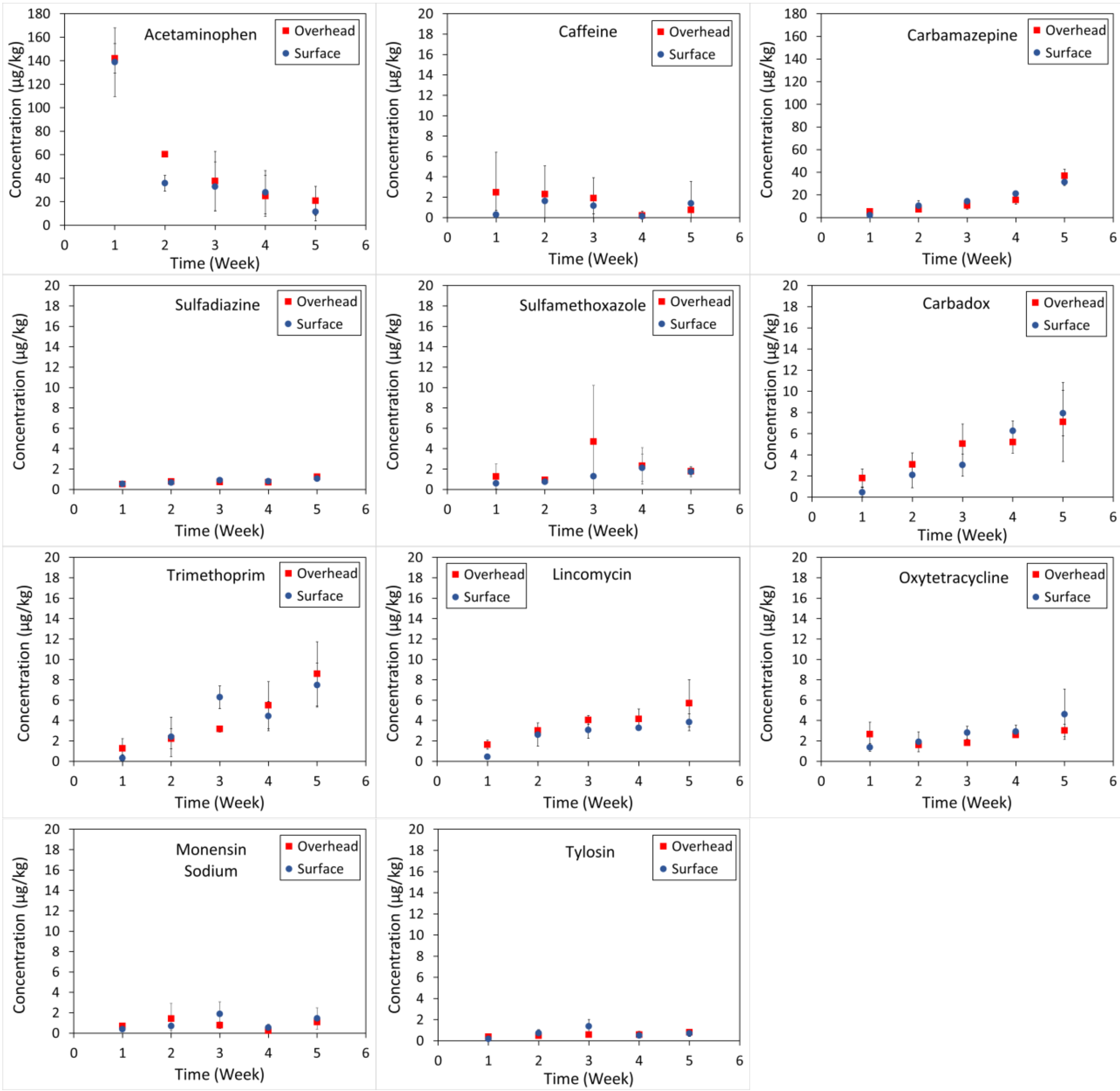


Figure 11. Pharmaceutical concentrations in lettuce roots for overhead and surface irrigated plants in Trial 2 (nominal pharmaceutical concentrations of 30 µg/L in irrigation water).

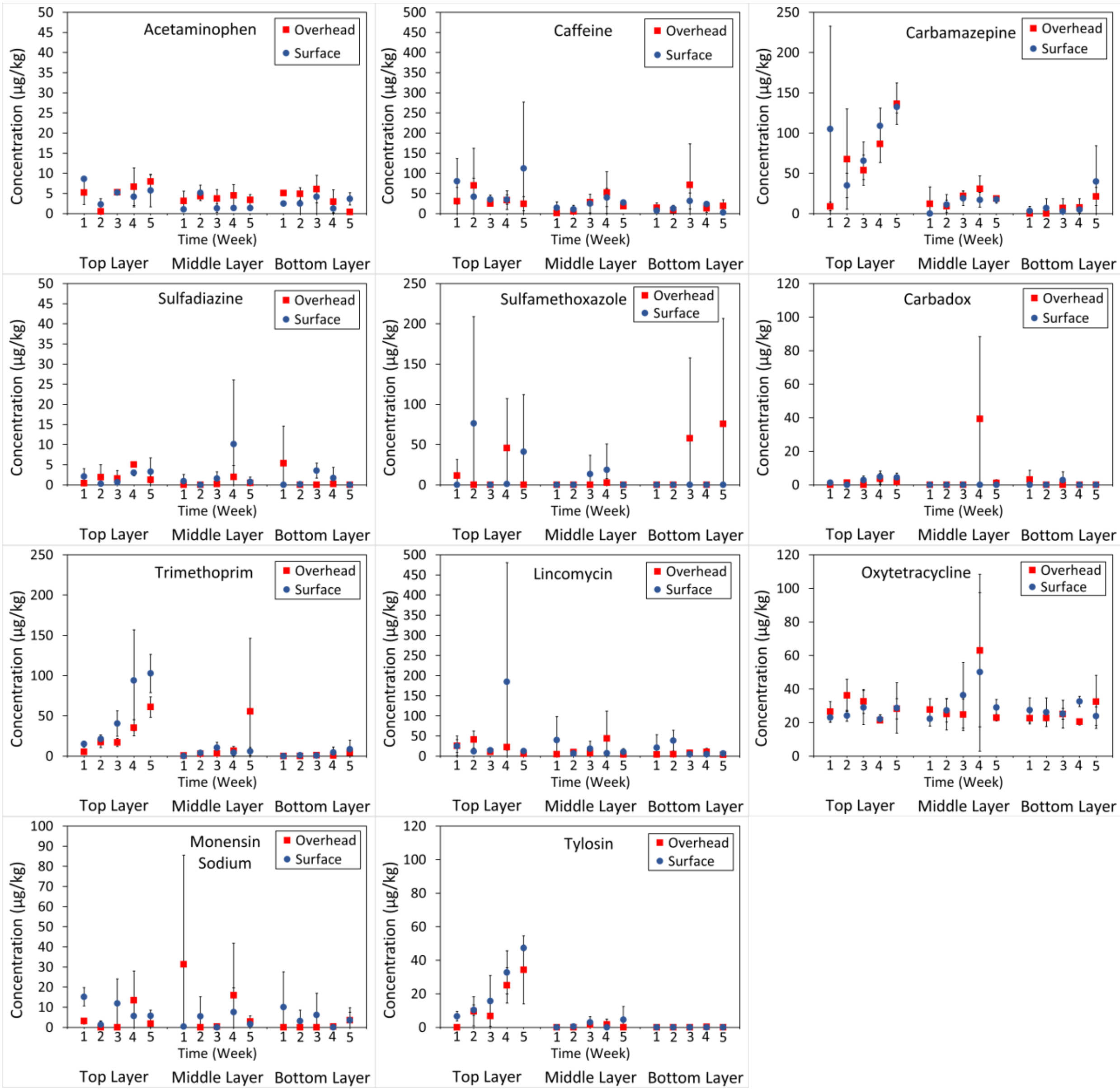


Figure 12. Pharmaceutical concentrations in soil for overhead and surface irrigated plants in Trial 1 (nominal pharmaceutical concentrations of 50 µg/L in irrigation water).

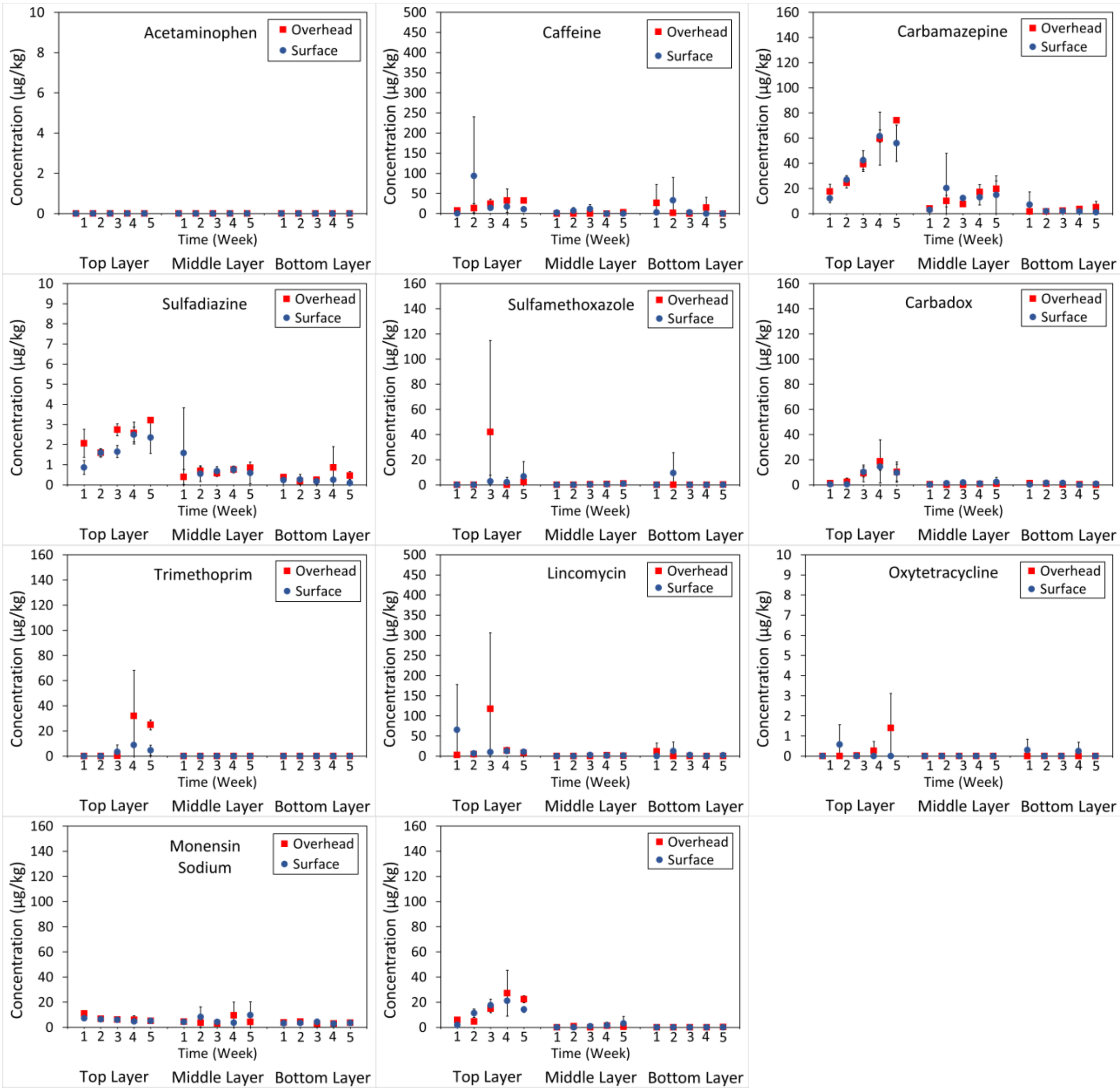


Figure 13. Pharmaceutical concentrations in soil for overhead and surface irrigated plants in Trial 2 (nominal pharmaceutical concentrations of 30 µg/L in irrigation water).

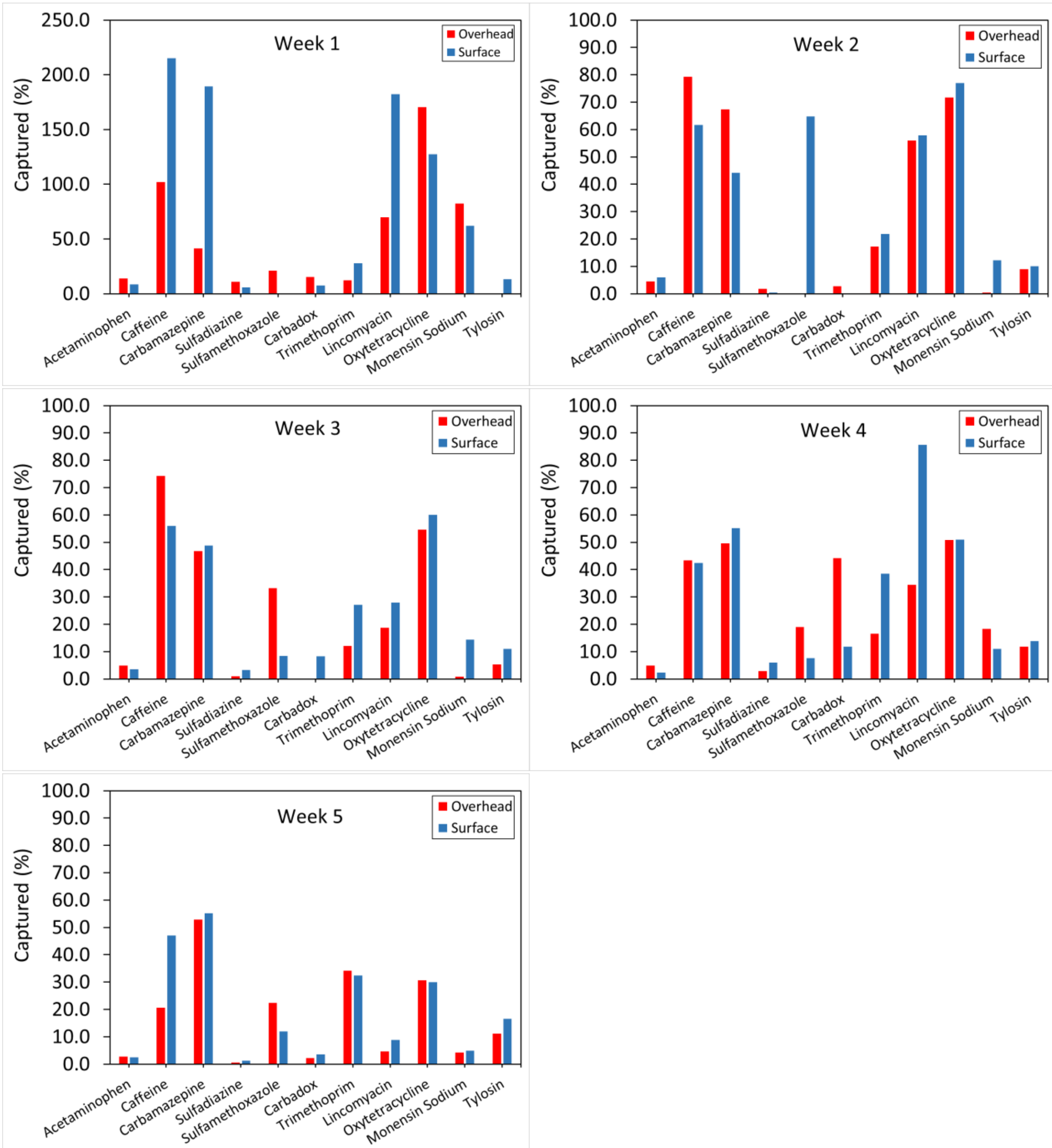


Figure 14. Total percent of each pharmaceutical recovered for the 50 µg/L Trial.

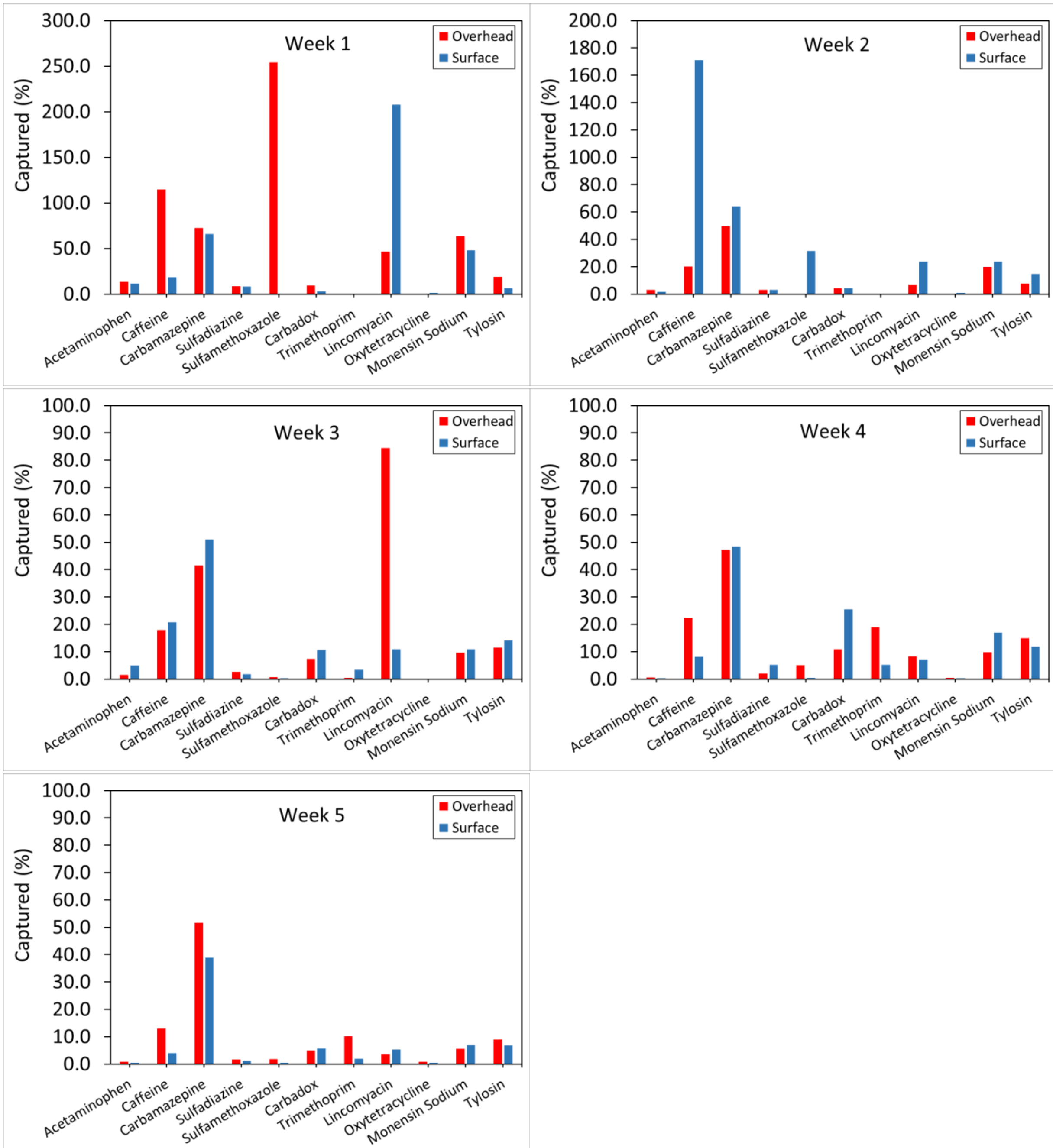


Figure 15. Total percent of each pharmaceutical recovered for the 30 µg/L Trial

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CHAPTER THREE: CONCLUSIONS AND FUTURE RECOMMENDATIONS

CONCLUSIONS

Pharmaceuticals can enter the environment through their use in animal agriculture and human medicine. Because pharmaceuticals are not fully metabolized, they can be excreted through waste. Pharmaceuticals have been widely detected in wastewater treatment effluents, surface water and groundwater. Agricultural irrigation accounts for the majority of fresh water for human use. Due to rising worldwide water stress and scarcity, using reclaimed water for agricultural irrigation is an increasingly popular way to conserve freshwater. Unfortunately, conventional wastewater treatment practices are inefficient at removing pharmaceuticals from final effluent. Therefore, reclaimed water can be contaminated with common pharmaceuticals that can possibly accumulate in crops. Irrigation method may play a large role in final concentration and mass of pharmaceuticals in crops.

This study investigated the uptake and accumulation of pharmaceuticals in overhead and surface irrigated lettuce. When lettuce was grown in a greenhouse under simulated overhead and surface irrigation using water containing pharmaceuticals, those having low lipophilicity, low molecular weight, and high water solubility (acetaminophen, caffeine, carbamazepine, sulfadiazine, sulfamethoxazole, and carbadox) were similarly concentrated in les shoots over time. However, pharmaceuticals with high lipophilicity, high molecular weight, and lower water solubility (monensin sodium and tylosin) exhibited higher concentrations in overhead as opposed to surface-irrigated lettuce shoots. Exceptions to this were trimethoprim and lincomycin which have large molecular weights, high water solubility, but low log K_{OW}. Both pharmaceuticals were more heavily concentrated in overhead-irrigated lettuce shoots likely because of diffusing into waxy leaf cuticles and binding with negatively charged leaf surface. Irrigation method played no role in final concentration of pharmaceuticals in roots or soil. Carbamazepine, trimethoprim, carbadox, and tylosin showed increased concentrations in the top layer of soil overtime indicating stronger sorption to loamy sand soils.

FUTURE RECOMMENDATIONS

Based on these findings, irrigation method could play a large role in final concentrations of pharmaceuticals in edible plants, depending on the chemical properties of pharmaceuticals and crop type. More research is necessary regarding different crops under both irrigation treatments, especially comparing root crops (i.e. carrots, radish, etc.) and fruit crops (i.e. tomatoes, cucumber, etc) pharmaceutical amounts. It is also necessary to understand how soil type plays a role in pharmaceutical uptake, so research should be done under both irrigation treatments with varying soil type and with possible soil amendments such as biochar to sorb contaminants. Degradation also plays a large role in final pharmaceutical amount, so more research should be done to help separate modes of degradation for specific pharmaceuticals.

Since using reclaimed water for agricultural irrigation is currently practiced in water scarce areas and its use is predicted to increase in the future, consideration on irrigation method and pharmaceutical type should be taken. Although it is difficult to predict final concentrations of pharmaceuticals in crops, the trends observed in this study can help inform growing practices and consumer washing practices to hopefully lower unintentional pharmaceutical exposure through food.

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