DISSERTATION

EFFECT OF GRAYWATER IRRIGATION ON SOIL QUALITY AND FATE AND TRANSPORT OF SURFACTANTS IN SOIL

Submitted by

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ABSTRACT

EFFECT OF GRAYWATER IRRIGATION ON SOIL QUALITY AND FATE AND TRANSPORT OF SURFACTANTS IN SOIL

While interest in and adoption of graywater reuse for irrigation has rapidly grown in recent years, little is known about the long-term effects of graywater irrigation. Concerns exist in relation to the presence of pathogenic organisms, fate of personal care products, and accumulation of salts. The purpose of this research was to evaluate the long-term effects of graywater irrigation to soil quality. The specific objectives were to evaluate the effects of graywater application on physical and chemical quality of soil, including surfactants, salts and boron accumulation, organic matter leaching and soil hydrodynamic properties in real environment in the field, in controlled environment in the greenhouse and column studies. In addition, fate and transport of surfactants in soil were investigated including how surfactant characteristics impacts mobility in soil of varying types. Graywater irrigation was found to significantly increase sodium in soil at households with graywater systems in place for more than five years; however SAR was not high enough in any of the sampling locations to raise concern about soil quality or plant health. There is a potential for salts, N, and B to leach through soil when graywater is applied for irrigation. A portion of the applied N is assimilated by plants, but leaching of N was observed. Graywater irrigation was also found to significantly increase surfactants in soil. Surfactants mainly accumulated in surface soil (0-15 cm) compared to depth soil. While surfactants have high sorption capacity due to their hydrophobic characteristics, they can be transported through soil if a large amount of water is applied. Among the surfactants measured in this study, AS and AES had the highest mobility. Mobility of surfactants in soil decreased when their number of ethoxylated groups increased. Adding organic matter to the soil increased sorption capacity of soil, as a result, more surfactants retained in the soil columns. Antimicrobials, including triclosan and triclocarban were detected in graywater irrigated areas only in surface soil samples, but not freshwater irrigated areas.

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And finally, my most earnest gratitude has to be extended to my wife, **Maryam**, for her prayers, love, constant support, and her patience at all times. Life is always a pleasant experience with her.

DEDICATION

This thesis is dedicated to the loving memory of my parents, whose unconditional love, tireless patience and constant encouragement during my childhood allowed me to realize my dreams for the future.

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ACRONYMS

AE: Alcohol Ethoxylates
AES: Alcohol Ethoxy Sulfates
ANOVA: Analysis of Variance
AS: Alkyl Sulfates
B: Boron
BOD: Biological Oxygen Demand
Ca: Calcium
CEC: Cation Exchange Capacity
Cl: Chloride
Cn: Carbon Chain Number
COD: Chemical Oxygen Demand
DOC: Dissolved Organic Carbon
EC: Electrical Conductivity
EO: Ethoxylated Groups
FAO: Food and Agriculture Organization
FW: Freshwater-irrigated
GW: Graywater-irrigated
K: Potassium
K_d: Adsorption Coefficient
K_{oc}: Partitioning Coefficient
LAS: Linear Alkylbenzene Sulfonates
Log K_{ow}: Octanol/Water Partition Coefficient
mM: Mili Molar
Mg: Magnesium
MPN: Most Probable Number
Na: Sodium
N: Nitrogen
ND: Not Detected
NH_4-N: Ammonia Nitrogen
NM: Not Measured
NOEC: No Observable Effect Concentration
NO_3-N: Nitrate Nitrogen
OM: Organic Matter
ORP: Oxidation-Reduction Potential
P: Phosphorus
PO_4-P: Phosphate Phosphorus
PW: Potable Water-irrigated
QAPP: Quality Assurance Project Plan

TCC: Triclocarban
TCS: Triclosan
TDS: Total Dissolved Solids
TN: Total Nitrogen
TOC: Total Organic Carbon
TP: Total Phosphorus
TSS: Total Suspended Solids
SAR: Sodium Adsorption Ratio
SDA: Soap and Detergent Association
WERF: Water and Environment Research Foundation

Chapter 1

INTRODUCTION

1.1. Background

As communities in arid and semi-arid regions throughout the United State and abroad are becoming interested in innovative approaches to new water management strategies, household graywater reuse for non-potable uses such as landscape irrigation and toilet flushing is gaining popularity. In a typical household, graywater (near 33 gallons per person per day) is nearly 50% of the total wastewater generated (Mayer et al. 1999). If used for irrigation of a typical residential landscape, it could supply about 30% of the demand, and with increasing emphasis on xeriscape in the semi-arid West, it has the potential to supply 100% of the irrigation demand in some areas.

By the most common definition in the U.S., graywater is wastewater that originates from laundry, bathtubs, showers and sinks and does not include wastewater from kitchen sinks, dishwashers and toilets (Eriksson 2002). The most simple reuse application for graywater is residential landscape irrigation because minimal treatment can be applied as compared to more complex system required for toilet flushing where human contact with water is likely and disinfection is therefore typically recommended.

Graywater has the potential to provide up to 33 gallons/person/day water for irrigation (Figure 1-1). A study conducted by the Soap and Detergent Association (SDA) in 1999 revealed that 7% of U.S. households were reusing graywater (NDP Group, 1999). Another study in the same year (Little, 1999) found that 13% of the households in Arizona used graywater for irrigation with the most utilized source being from clothes washers (66%). This number is likely now even larger as many states have begun to allow graywater reuse over the last several years including

Arizona, New Mexico, Texas, Nevada, and Utah in the southwestern United States (ADEQ 2003).

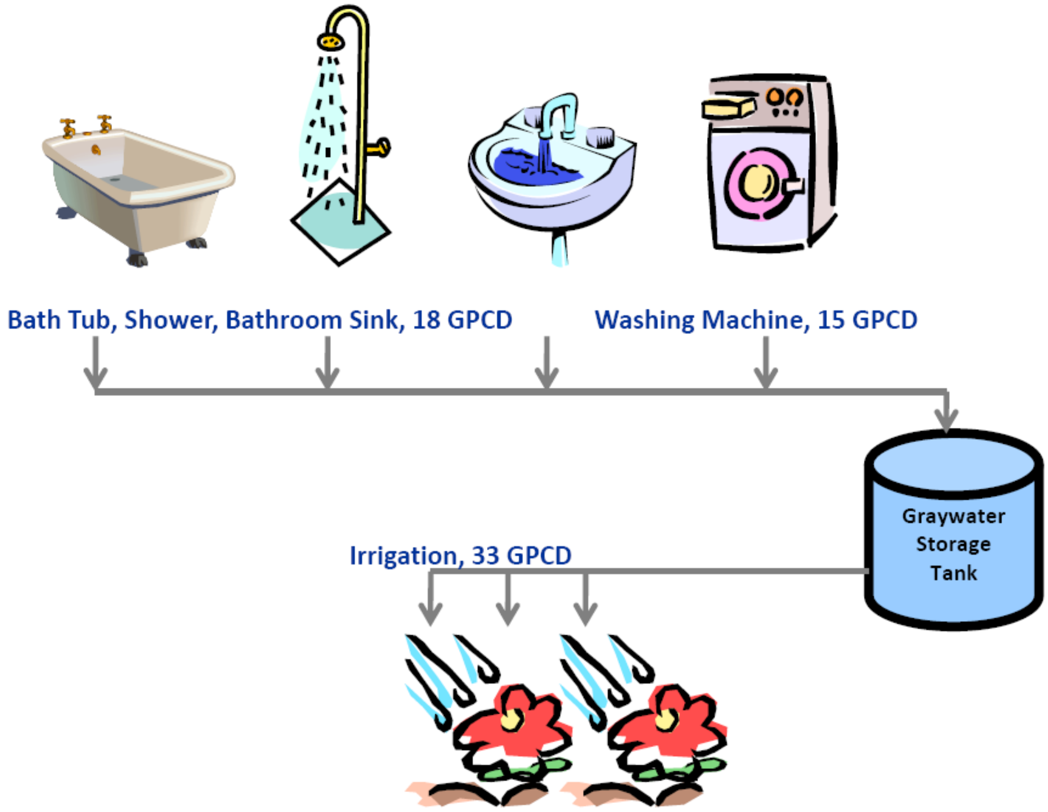


Figure 1-1. Potential graywater reuse for landscape irrigation (GPCD: Gallon per capita per day; Source: American Water Works Association and AWWA Research Foundation)

1.2. Concerns Associated with Reuse of Graywater for Irrigation

Although reuse of graywater for landscape irrigation is gaining momentum, limited scientific data is available on its impacts on soil properties (Misra and Sivongxay 2009). Graywater contains a complex mixture of chemicals used in household products including surfactants, bleach, dyes, enzymes, fragrances, builders etc (Table 1-1). When graywater is reused for irrigation, constituents of concern include pathogens and/or viruses, organic content, nutrients (nitrogen (N) and phosphorus (P)), metals, salts, boron (B), and xenobiotic organic compounds (XOCs) mainly including surfactants and antimicrobials (Dixon et al. 1999).

Table 1-2. Typical Household Graywater Quality.

Source	pH	Chemical Oxygen Demand (mg L^{-1})	Total Nitrogen (mg L^{-1})	Total Phosphorus (mg L^{-1})	Sodium Adsorption Ratio	Anionic Surfactants (mg L^{-1})	Reference
Domestic (unspecified)	8.1 ± 0.1	-	19 ± 1.6	31 ± 6	5.9	34 ± 8.2	Wiel-Shafran et al. (2006)
Bath, dish washing and laundry	6.3 - 7.0	702 - 984	25.0 – 45.2	1.72 ± 27	-	4.7 – 15.6	Gross et al (2007)
Shower and laundry	6.7 - 7.6	278 - 435	-	0.24 - 1.2	4.2 - 5.8	-	Finely et al. (2009)
Shower, Hand-wash, bath, laundry	6.3 - 8.1	310 - 580	21.8 - 73.8	4.4 - 16.4	2.3 - 4.1	4.6 - 16.7	Current Study

Surfactants have been identified as components of graywater that can cause water repellency and reduce soil hydraulic conductivity (Lado and Ben-Hur, 2009; Travis et al. 2008; Wiel-Shafran et al. 2006). In addition, surfactants may affect the mobility and degradation of hydrophobic organic compounds in soil or sediment (Edwards et al. 1994; Tiehm 1994). Other potential detrimental effects of graywater reuse include soil aggregate dispersion from sodium accumulation (Misra and Sivongxay, 2009); microbial risks (Gross et al. 2007a); and enhanced contaminant transport (Graber et al. 2001). B is another concern because it is toxic to plants when presents in irrigation water (Mahler 2009; Blevins and Lukaszewski 1998). N and P present in graywater may be beneficial for plant growth, but may be a concern if transported to groundwater. A previous study by Pinto et al. (2009) showed no significant differences in total N and P in soils irrigated with graywater compared to soil irrigated with freshwater.

1.3. Graywater Impacts to Soil Quality

Application of graywater for irrigation may impact soil chemistry. Graywater may contain elevated sodium from powder detergents containing anionic surfactants (Jeppesen 1996). A study conducted by the City of Los Angeles (1992) showed that sodium increased in soil after irrigation with graywater; however, negative effects on plant growth and quality of landscape plants were not observed. Salts are a concern for reuse water and their accumulation has been problematic at some sites irrigated with reclaimed wastewater (Qian and Mecham, 2005). Graywater may contain elevated sodium (Jeppesen, 1996). Boron is another concern because it is toxic to plants when presents in irrigation water at 1.8 mg L^{-1}or more (Mahler, 2009; Blevins and Lukaszewski, 1998). However, research to date has not reported effects of graywater irrigation to boron concentration in soil.

A large component of the organic compounds in graywater is surfactants. Surfactants are used in household cleaning products, cosmetics, detergents, lubricants (and other miscellaneous industrial applications). Surfactants present in graywater are of concern due to their potential toxicity on plants and soil organisms, effects on soil physical property and their effects on mobility of hydrophobic compounds in the soil. In addition, surfactants applied in graywater may be transported to groundwater. Research on surfactants, antimicrobials, dyes and enzymes is limited. While some researchers have shown potential negative impacts of elevated surfactants concentration to soil, the fate of surfactants after applied to soil is largely unknown. These negative impacts may include decrease in hydraulic conductivity and reduction in capillary rise (Abu-Zreig 2003 and Wiel-Shafran et al. 2006). Some recent studies on graywater indicated accumulation of surfactants up to 680 μg kg^{-1} as anionic surfactants when soils were irrigated with graywater and possible alteration of soil properties such as causing higher water repellency

(Travis et al. 2010). The direct phytotoxic effects of surfactants will be dependent on the rate of degradation of the surfactants as well as the toxic threshold of individual plants (Garland et al. 2000). Surfactants have been shown to have toxic effects on stream microorganisms with the lowest no observed effect concentrations (NOEC) was reported for a stream mesocosm at concentrations between 0.22-0.29 mg L^{-1} surfactant. Toxicity thresholds have not been developed for soil organisms. Little information is available to date on the fate of surfactants after application in graywater and further study is needed in this area to address concerns.

Another component of concern in personal care products is antimicrobials, such as triclosan (TCS) and triclocarban (TCC). Results from a preliminary assessment conducted by Canadian Environmental Protection Agency (CEPA; 2012) concluded that current levels of TCS in personal care products do not pose a risk to human health. However as toothpastes, soaps and other items are rinsed off and washed down the drain, the amount of TCS that is released into the environment can affect plants and animals in lakes, streams and rivers. The main concern is linked to antibacterial resistance. However, based on available information, there is no clear link between use of products containing TCS and antibacterial resistance (Chemical Substances, Chemicals Management Plan, 2012). The presence of TCS and TCC have not been determined in soil irrigated by graywater to date and more information is needed to determine the risk associated with antimicrobials in graywater.

1.4. Graywater Impacts to Groundwater Quality

Graywater constituents may impact groundwater quality in addition to soil quality if the constituents are transported to groundwater. Nutrients and synthetic organics (surfactants, antimicrobials, etc.) are a concern for environmental quality and human health. This is of particular concern when graywater is applied at a rate higher than required for irrigation, which is often done when graywater application is controlled by a homeowner. Studies to date have not addressed this concern. However, reclaimed water reuse for irrigation has been largely applied for decades and many studies have been conducted on the leachability of various constituents after application of recycled water for irrigation. The review provided here will focus on studies on reclaimed wastewater since literature is not available to date on leaching of chemical constituents when graywater is applied for irrigation. While some of these studies suggested that reclaimed wastewater may enhance soil quality by adding nutrients and OM to the soil, researchers recommended risk assessment to be conducted prior to irrigation with reclaimed water to ensure safe application of treated wastewater (Candela et al. 2007; Kalavrouziotis et al. 2008; Gharbi Tarchouna et al. 2010; Xu et al. 2010; Chavez et al. 2011). Leaching of nitrates is one of the greatest threats to groundwater quality arising from reclaimed water irrigation due to its high solubility (Bond et al. 1998; Hermon et al. 2006). Candela et al. (2007) reported higher values of nitrate in groundwater samples under reclaimed wastewater irrigated areas compared to areas irrigated with potable water. Graywater contains less N than residential wastewater not only because it does not contain urine, which is the main source of nitrogen, but also because usually kitchen water is excluded from graywater. Leaching of nitrogen from graywater application may be of lower concern than reclaimed wastewater because nitrogen concentrations are lower in graywater (0.6-21 mg L^{-1} total nitrogen (TN) if kitchen water is excluded; Eriksson et al. 2002) than reclaimed water, except when the wastewater treatment process includes denitrification. Leaching of B and salts are other concerns associated with reuse of reclaimed wastewater for irrigation. Stewart et al. (1990) observed long-term changes in soil pH as a result of displacing cations or excessive leaching of them into deeper horizons caused by reclaimed wastewater irrigation. In another study, no B retention was observed in soil-turf filters irrigated with reclaimed wastewater, which indicates high leaching potential for B (Anderson et al. 1981). Because B in water is not a human health concern, leaching of B after applied in irrigation water is not viewed as a major concern. While little is known on fate and leaching of personal care products, few studies raised the concern over leaching of xenobiotic compounds and emerging contaminants into groundwater due to reclaimed wastewater application (Pedersen et al. 2005; Chefetz et al. 2008; Xu et al. 2009).

1.5. Public Health Concerns

Public health concerns about graywater exist with respect to the potential for human exposure to pathogenic organisms after graywater is applied for irrigation. A number of studies have inferred fecal contamination of graywater via the presence of indicator organisms (e.g., Novotny, 1990; Rose et al., 1991; Christova-Boal et al., 1996; Casanova et al., 2001; and Ottoson et al., 2003). A primary concern is the possibility of graywater irrigation being a pathway for the spread of human diseases. While it is well established that graywater contains indicator organisms, the fate of pathogens after graywater application is not well understood and their persistence could result in human health risks.

1.6. Graywater Impacts to Soil Hydrodynamics

Capillarity is the primary force that enables the soil to retain water, as well as to regulate its movement in an unsaturated soil. Capillarity in soil can have both beneficial and detrimental effects. It is the main mechanism by which plants can pull water from below the root zone. However, it may contribute to the accumulation of salts in the soil causing the dry land salinity. Scientific data regarding the effects of surfactants on the hydro-physical properties of soils receiving graywater and on the capillary rise in particular is very limited (Abu-Zreig et al. 2003). Surfactants reduce surface tension of water by accumulating at the liquid/gas or solid/liquid interface (Kuhnt, 1993). As described by Young-Laplace equation, capillary rise is directly related to the surface tension:

(1)

$$h \approx -\frac{2\sigma cos\phi}{r\rho g}$$

Where:

h: capillary rise

σ: surface tension of the liquid

ϕ: contact angle of the liquid-air interface

ρ: is the density of the liquid, g

g: gravity force and

r: capillary radius

As a result, it is expected that surfactants, which are present in graywater, reduce the surface tension of water in soil pores and cause lower capillary rise in soil. This change in capillarity will affect movement of water in unsaturated zone.

1.7. Graywater Impacts to Plant Health

Changes in soil chemistry resulting from graywater application may affect plant health. Some studies have shown negative impacts to plant health resulting from graywater irrigation, while others have shown that graywater constituents may have a positive effect on plant health (City of Los Angeles, 1992; Rianallo et al. 1988; Bubenheim et al. 1997). Further research is required to adequately understand the effects of graywater irrigation on plant health.

1.8. Fate and Behavior of Surfactants in the Environment

Surfactants are compounds which lower the surface tension of a liquid, increasing the contact between the liquid and another substance. The term is a compound of "surface acting agent," referring to the fact that a surfactant interacts with the surface of a liquid to change its properties. There are a wide variety of surfactants including detergents, wetting agents, emulsifiers, foaming agents, and dispersants, which work with oil, water, and an assortment of other liquids. Surfactants are usually organic compounds which contain both hydrophobic groups (their tails) and hydrophilic groups (their heads; Figure 1-2). As a result their molecules are amphiphilic, meaning they contain both a water insoluble (or oil insoluble) component as well as a water soluble component.

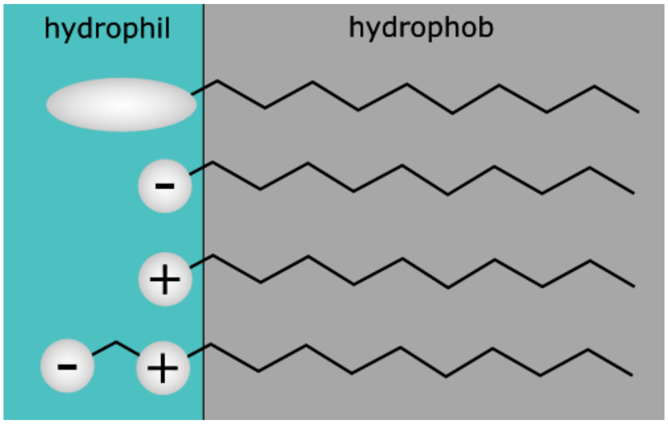


Figure 1-2. General Structure of Surfactants

Surfactants can have a cationic, anionic or neutral head (Figure 1-2). If the head of a surfactant carries a negative charge it is called anionic, if it carries a positive charge it is called cationic and if it has no charge it is called non-ionic. A list of major anionic, cationic and non-ionic surfactants is presented in Table 1-2.

Surfactants are produced in large volumes globally and used in households as detergents and personal care products resulting in potential "down the drain" discharge of these compounds into residential wastewater. As a result, a large component of the organic content in graywater is surfactants. For example total anionic surfactants have been found to range from 4-34 mg L^{-1} in graywater generated from a typical household (Table 1-1). In this research, we will focus on linear alkylbenzene sulfonates (LAS), alkyl sulfates (AS), alkyl ethoxysulfates (AES) as the most common anionic surfactants and alcohol ethoxylates (AE) as the most consumed non-ionic surfactants in personal and household cleaning products.

Table 1-2. Some Commonly Used Surfactants of Each Type

Type	**Some of the Common Surfactants**
Anionic	Alkyl benzene sulfonates, Alkyl ether sulfates, Alkyl sulfates, Alkyl ether phosphate and Alkyl carboxylates
Cationic	Alkyltrimethylammonium salts, Dioctadecyldimethylammonium bromide, Benzalkonium chloride, and Cetylpyridinium chloride
Non-ionic	Fatty alcohols, Polyoxyethylene glycol alkyl ethers and Polyoxypropylene glycol alkyl ethers

1.8.1. Surfactants Analyzed in this Research

LAS, AS/AES and AE are all produced in large volumes globally resulting in potential “down the drain” discharge of these compounds into residential wastewater. These surfactants were those chosen to study for this research. The annual consumption volumes of LAS, AS and AES in North America were estimated to be 700, 230, and 1083 million pounds respectively (Figure 1-3; Modler et al. 2004). In addition, 453 million pounds of AE was consumed in North America in 2003 (Figure 1-3; SRI, 2004). These compounds are the major cleaning agents found in detergents and personal care products result in considerable level of these chemicals found in graywater.

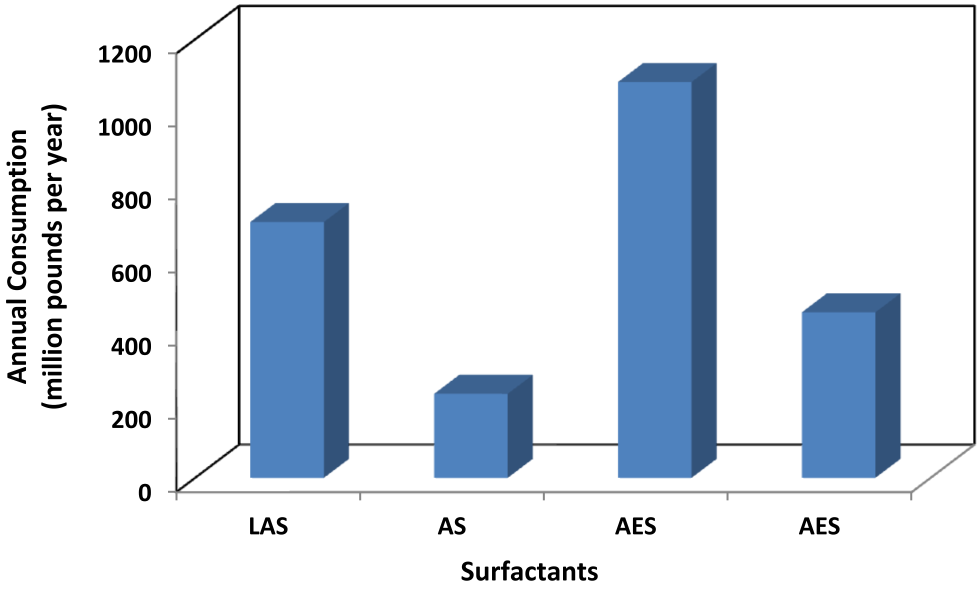


Figure 1-3. Annual consumption of surfactants in North America in 2003 (Modler et al. 2004 and SRI 2004)

1.8.1.1.Linear Alkylbenzene Sulfonates (LAS)

LAS is a specific mixture of closely related isomers and homologues generated in the manufacture of the raw material Linear Alkyl Benzene (LAB). These isomers contain an aromatic ring sulfonated at the "para" position and attached to a linear alkyl chain at any position except the terminal carbons as described in Figure 1-4 (Schönkaes, 1998; Cavalli et al. 1999; Valtorta et al. 2000). The linear alkyl chain typically has 10 to 13 carbon units, approximately in the mole ratio of C10:C11:C12:C13=13:30:33:24 (Feijtel et al. 1999; Cavalli et al. 1999; Valtorta et al. 2000). LAS is the most widely used anionic surfactants found in laundry powders, laundry liquids, dishwashing products and all-purpose cleaners (HERA 2009a). As a result LAS can be found in the levels of 2.8 to 9.1 mg L^{-1} in raw sewage and up to 34 mg L^{-1} in graywater (Matthijs et al. 1999; Table 1-1).

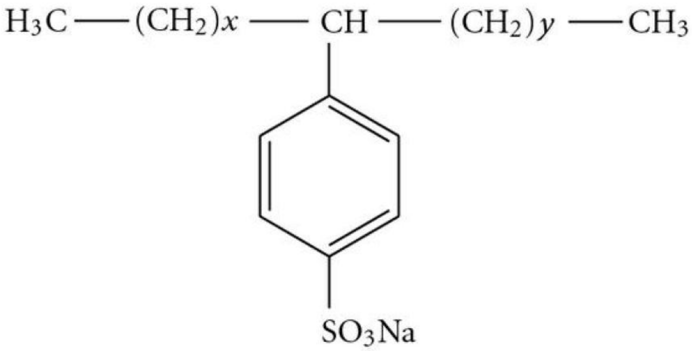
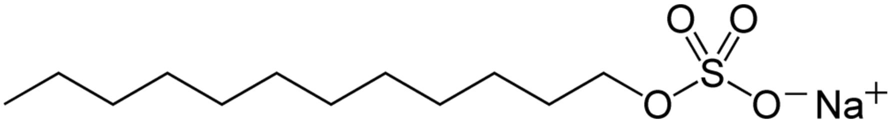


Figure 1-4. Linear Alkylbenzene Sulfonates (Alkyl Chain: C10-C13; x+y: 7-10)

1.8.1.2.Alkyl Sulfates (AS)

Alkyl Sulfates (AS) are a widely used class of anionic surfactants found in household cleaning products, personal care products including toothpaste and shampoos, hand washes and other personal cleaning products, institutional cleaners and industrial cleaning processes (HERA 2002). The Alkyl Sulfate family is defined as linear-type primary alkyl sulfates containing AS components of basic structure $C_nH_{2n+1}SO_4$ M, where n=12-18 and M= sodium, ammonium or triethanolamine. Of note is sodium neutralized AS are by far the most predominant grades (HERA 2002). The principle linear structure of C_{12} AS for example is shown in Figure 1-5. Of the AS used in cleaning products globally, a preliminary estimate gives 85-90% derived from even carbon numbered linear alcohols (C_{12-14} and C_{16-18}), with the remaining 10-15% derived from odd and even carbon numbered essentially linear-oxo alcohols.



$CH_3{-}(CH_2)_{11}{-}O{-}SO_3{-}Na$

Figure 1-5. Alkyl Sulfate (Linear Isomer with Alkyl Chain: C_{12})

1.8.1.3.Alcohol Ethoxysulfates (AES)

Alcohol ethoxysulfates (AES), also known as alkyl ethersulfates, are a widely used class of anionic surfactants used in toothpaste and shampoos, hand washes and other personal cleaning products, institutional cleaners and industrial cleaning processes (HERA 2003). The alcohol ethoxysulfate family is defined for as linear-type primary alcohol ethoxysulfates containing AES components of basic structure C_nH_{2n}+$O(C_2H_4O)_mSO_3X$ where n=10-18 and m = 0-8 and X = sodium, ammonium or triethanolamine (TEA). Of note is sodium salts of AES are the most commonly used grades (HERA 2003). C_{12} through C_{15} are the most produced grades in the feedstock. Typically, 40-90% of the carbon chains are linear, the remainder being mono-branched 2-alkyl isomers, predominantly 2-methyl. For example, the linear structure of AES is shown in Figure 1-6. The number of ethoxyl groups, n, ranges from 0-8 moles EO per mole alcohol however, the average value for n is 0-3 (HERA 2003).

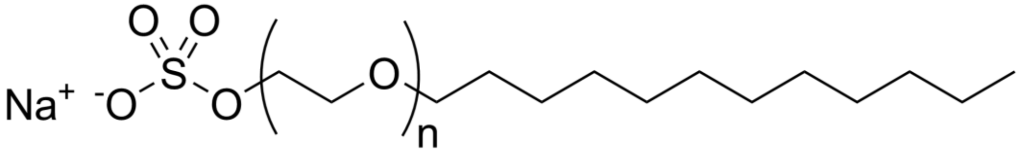
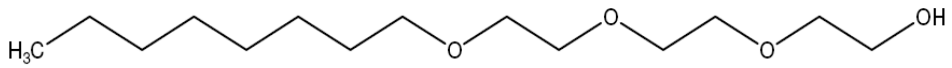


Figure 1-6. Linear Structure of AES (n= number of ethoxyl groups)

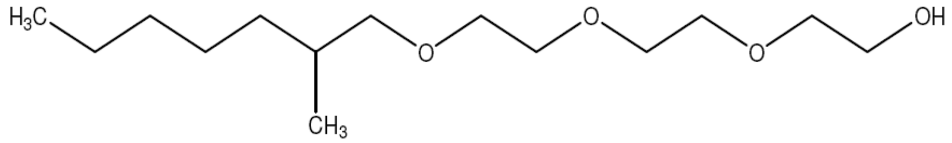
1.8.1.4.Alcohol Ethoxylates (AE)

Alcohol ethoxylates (AE) are a widely used class of non-ionic surfactants. Desirable characteristics of AE including rapid biodegradation, low to moderate foaming ability, superior cleaning of man-made fibers and tolerance of water hardness made it as the most commonly used non-ionic surfactants (HERA 2009b). Significant quantities of AE are converted to alcohol ethoxysulfates (AES) with the remaining AE used primarily in household laundry detergents

(HERA 2009b). The AE family is defined as the basic structure of $C_{x-y}AE_n$ where x-y indicates the range of carbon chain units. The most commonly used AE in household detergents contain carbon unit range between C_8 to C_{18} (HERA 2009b). Two principle structures of AEs present in household cleaning products are presented in Figure 1-7.



Linear AE (C_8EO_3)



Essential linear, methyl branched AE (C_8EO_3)

Figure 1-7. Two principle structures of AEs present in household cleaning products

1.8.2. Chemical Characteristic of Surfactants

An essential property of surfactants is their ability to form micelles in solution. This property is due to the presence of both hydrophobic and hydrophilic groups in a surfactant molecule. It is the formation of micelles in solution that gives surfactants their detergency and solubilization properties. When dissolved in water at low concentration, surfactant molecules exist as monomers. At higher concentrations, the system's free energy can be reduced due to aggregation of the surfactant molecules into micelles with the hydrophobic groups located at the center of the micelle and the hydrophilic head groups towards the solvent. The concentration at which this occurs is known as the critical micelle concentration (CMC) (Haigh, 1996). Nonionic surfactants have lower CMC levels than anionic and cationic surfactants (Table 1-3).

Table 1-3. Critical Micelle Concentration of Surfactants

Compound	**CMC (mM) (distilled water)**	**Reference**
C12LAS	1.1	Tolls and Sijm (1995)
C13LAS	0.46	
C12EO4	0.064	
C12EO8	0.11	
NPEO10	0.094	Kibbey and Hayes (2000)
NPEO12	0.057	Brix et al. (2001)

At concentrations above the CMC level, surfactants have the ability to increase the solubility of hydrophobic organic compounds compared to water alone (Figure 1-8). Surfactants may affect the mobility and degradation of hydrophobic organic compounds in soil or sediment (Edwards et al. 1994; Tiehm 1994). Aronstein et al. (1991) found that the extent of phenanthrene biodegradation was markedly increased at nonionic surfactant concentrations of 10 μg kg^{-1} soil in both a mineral and organic soil, despite lack of desorption enhancement in the organic soil. Ying et al. (2005) also found that small percentages (> 1%) of surfactants in water could mobilize triazines in contaminated soils. The fate of surfactants in the environment depends on sorption to soil, biodegradation, precipitation and leaching potential (Ying et al. 2006).

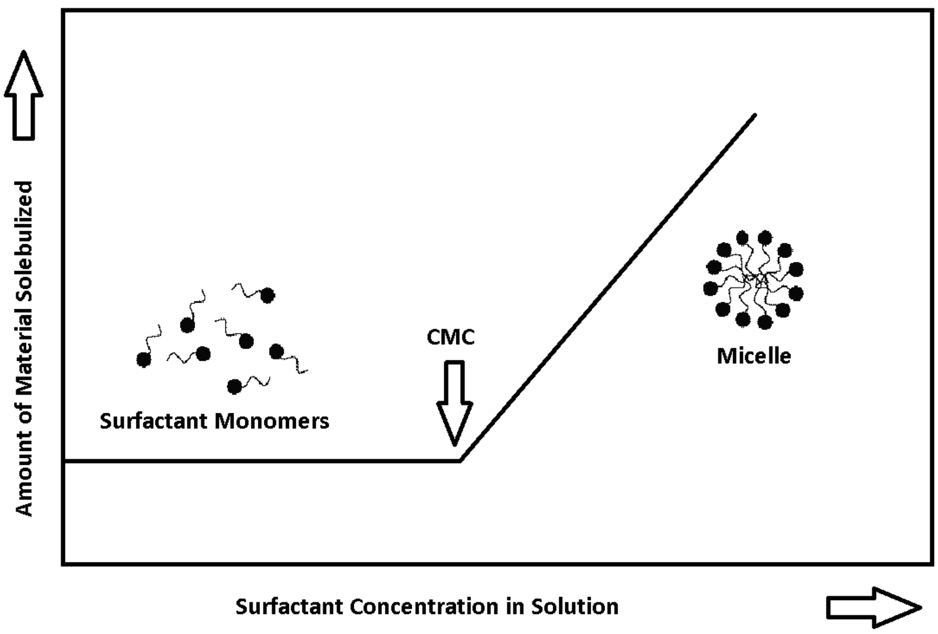


Figure 1-8. Solubilization of Materials as a Function of Surfactant Concentration

1.8.3. Sorption of Surfactants

Sorption of surfactants onto sediment/soil depends on many factors including their physiochemical properties, soil nature and environmental parameters. The information from sorption process of surfactants can be used to estimate the distribution of the surfactant in different environmental compartments (sediment/soil and water). Sorption data can also be used to estimate the bioavailability of the surfactant. Furthermore, sorption has a substantial influence on the degradation of the surfactant in the environment.

Due to their chemical features, surfactant molecules may sorb directly onto solid surfaces or may interact with sorbed surfactant molecules. The sorption mechanism is dependent on the nature of the sorbent and the surfactant concentration (Adeel and Luthy 1995; Brownawell et al. 1997; Fytianos et al. 1998; Ou et al. 1996). At higher concentrations, sorption may entail the formation of more structured arrangements including the formation of monomer surfactant clusters on the surface or a second layer, for which these arrangements may be governed mainly by interactions between hydrophobic moieties of the surfactant molecules. Therefore, two stage sorption isotherms (Figure 1-9) have been reported for nonionic surfactants NPE and AE and anionic LAS although the sorption behavior is different for nonionic and anionic surfactants (Adeel and Luthy 1995; Brownawell et al. 1997; Fytianos et al. 1998; Ou et al. 1996).

The sorption of LAS on natural soils had two stages: linear and exponentially increasing isotherms (Ou et al. 1996). At low LAS concentration (< 90 µg/mL), the sorption isotherms were linear and the adsorption coefficient (K_d) ranged from 1.2 to2.0. At high concentrations (> 90 µg/mL), cooperative sorption was observed and the sorption amount of LAS increased exponentially with the increasing of LAS concentration in solution. This enhanced sorption of LAS on soils was also observed by Fytianos et al. (1998). In an actual soil or aquatic environment where LAS levels are rather low, the LAS sorption ability of a soil or sediment is very weak. Given the concentration of LAS found in graywater (<34 mg L^{-1}), adsorption of LAS on soil receiving graywater is expected to be weak and follow the linear stage as well. Of note is adsorption of LAS depends on soil characteristics and may vary based on clay and organic contents of the soil (Ou et al. 1996).

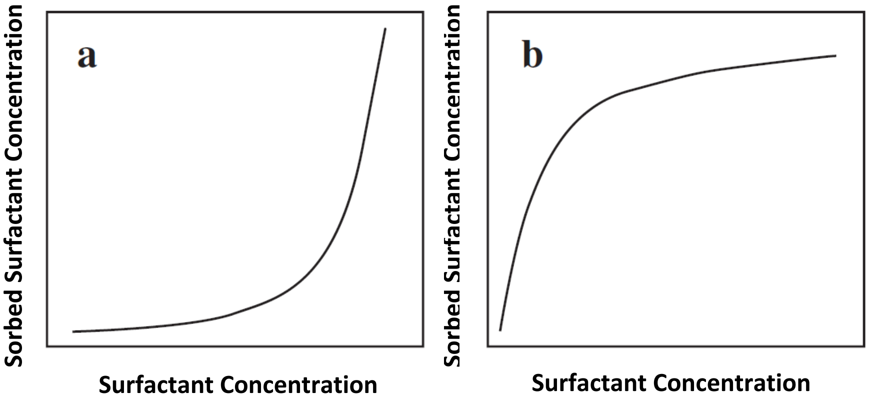


Figure 1-9. Sorption Isotherm of Surfactants (a: Anionic, b: Nonionic; Ying et al. 2006)

Marchesi et al. (1991) reported that AS adsorbs to the sediment via a hydrophobic interaction however adsorption of AS on kaolinite was negligible compared with the adsorption of the C_{10}-C_{13} homologues of LAS studied previously. Of note is adsorption of AS increases when carbon chain length increases (SIAM 2007).

In contrast, the sorption of a nonionic surfactant reached a maximum on the solid surface when the solution is near or just at the critical micelle concentration of the surfactant. The decreased sorption of nonionic surfactants (APEs and AEs) on sediment at higher concentrations was observed (Adeel and Luthy 1995; Kibbey and Hayes, 2000). AE adsorption to soil and sediments depends upon both the properties of AE homologues and the soil and sediments characteristics, but it is higher than LAS and AES in general (Nakis and Ben-David 1985; Yuan and Jafvert 1997; HERA 2002, 2003, 2009a,b). As a result, AE adsorption may vary based on organic and clay content of the soil. While there is little available data on biodegradation of AE in soil, AE is expected to have less leaching potential given its concentration in graywater and high adsorption capacity.

1.8.4. Biodegradation of Surfactants

Degradation of surfactants through microbial activity is the primary transformation occurring in the environment (Ying et al. 2006). Biodegradation is an important process to treat surfactants in raw wastewater in wastewater treatment plants (WWTPs), and also enhances the removal of these surfactants in the environment, thus reducing their impact on biota. During biodegradation, microorganisms can either utilize surfactants as substrates for energy and nutrients or co-metabolize the surfactants by microbial metabolic reactions. There are many chemical and environmental factors that affect biodegradation of a surfactant in the environment. The most important influencing factors are chemical structure, and physicochemical conditions of the environmental media. Different classes of surfactants have different degradation behavior in the environment (Table 1-4). Most of the surfactants can be degraded by microbes in the environment, although some surfactants such as LAS may be persistent under anaerobic conditions.

Table 1-4. Biodegradability of Surfactants in the Environment (Ying 2006 and references therein)

Surfactants	Aerobic Condition	Anaerobic Condition
LAS	Degradable	Persistent
SAS	Readily degradable	Persistent
Soap	Readily degradable	Readily degradable
AS	Readily degradable	Degradable
AES	Readily degradable	Degradable
Cationic Surfactants	Degradable	Persistent
AE	Readily degradable	Degradable

According to OECD standards on testing and assessment:

Readily degradable: 70 to 100% in 28 days

Degradable: 20-70% degradation in 28 days

Persistent: 20%> degradation in 28 days

LAS can be degraded under aerobic conditions by a consortium of aerobic microorganisms and attached biofilms (van Ginkel, 1996; Yadav et al. 2001 and Takada et al. 1994). Given the highly variable adsorption of LAS and its unlikely biodegradation under anaerobic conditions, fate and transport of LAS is highly variable in soil and depends on environmental conditions (aerobic or anaerobic) and soil characteristics. As a result potential penetration of LAS into deeper soil cannot be ignored under anaerobic conditions.

The available data on biodegradability of AS indicates that AS with all chain-lengths are readily and ultimately biodegradable under aerobic and anaerobic conditions (BUA 1996). Once released into environment, relevant fractions of the alkyl sulfates with higher chain length are expected to adsorb onto soil and sediments (SIAM 2007). However, due to the high degree of biodegradability, accumulation in soils is not expected.

Aerobic biodegradability of AES is well-established (Swisher 1987, Holt et al. 1992). In addition, anaerobic biodegradability of the structurally related alcohol ethoxylates and alkyl sulfate is well proven. As a result, ultimate anaerobic degradation of AES is likely to occur (Steber and Berger, 1995). Based on the study conducted by Sharvelle et al. (2007) complete degradation of the AE using biological waste treatment would be limited and only 40–70% of the surfactant molecules were readily biodegradable (Sharvelle et al. 2007).

1.9. Fate and Transport of Surfactants in Soil after Graywater Irrigation

Research on fate and transport of surfactants in soil irrigated with graywater is limited. Some recent studies on graywater indicated accumulation of surfactants in soil after graywater application. Travis et al. (2010) reported total surfactants concentration as 0.68±0.39, 0.15±0.06 and 0.53±0.14 mg/kg in sand, loam and loess irrigated with raw graywater respectively. However, Wiel-Shafran et al. (Shafran et al. 2005 and Wiel-Shafran et al. 2006) in two studies, reported up to 60 mg/kg and 30±7.2 mg/kg accumulation of anionic surfactants in soil receiving graywater using the methylene blue active substances (MBAS) method, much higher that than the concentrations reported by Travis et al. (2010). However, of note is that Wiel-Shafran (2006), also reported surfactants in control areas irrigated with freshwater between 5 and 6 mg kg^{-1}. These values seem excessively high, given that biosolids amended soil has been found to contain 16 and 53 mg/kg of linear alkylbenzene sulfonate (LAS) immediately after biosolids application containing 7000 and 30,200 mg/kg respectively (Berna et al. 1989). Of note is MBAS which has been used in most of these studies is an indirect measurement of surfactants and is less sensitive than LC/MS analysis.

One of the main mechanisms responsible for surfactant mobility is sorption. It is expected that surfactants will adsorb to soil particles due to their intensive surface activity and hydrophobic characteristic (Ying 2006 and Boluda-Botella et al. 2010). However, the sorption of surfactants (e.g. LAS) was reversible according to data reported by Boluda-Botella et al. 2010. As a result, anionic surfactants may reach deeper soil if sufficient water is applied to the soil. In addition, surfactants can be biodegraded in soil depends on environmental conditions and surfactants characteristics (Ying 2006 and Sharvelle et al. 2007). For example, Sharvelle et al. (2007) reported that for studied surfactants including anionic (sodium laureth sulfate - SLES) and nonionic surfactants (polyalcohol ethoxylate - PAE), only a fraction of the organic carbon was found to be readily biodegradable. In addition, according to Ying (2006), some surfactants (e.g. LAS) are persistent to anaerobic biodegradation and the anaerobic conditions in deep soil may favor the existence of surfactants in the depth samples after they penetrate to the deeper soil (Ying 2006).

1.10. Fate and behavior of Antimicrobials in the Environment

Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol; TCS) and triclocarban (3,4,4'-trichlorocarbanilide; TCC) are popular antimicrobial agents contained in a variety of consumer products of daily use including hand soaps. While TCC is added mostly to antimicrobial soaps, use of triclosan is broader and includes applications in antibacterial mouthwash and toothpaste, as well as in household items such as plastic cutting boards, sports equipment, textiles and furniture (Bester 2003, Sabaliunas et al. 2003; U.S. EPA 2003). Antimicrobial soaps typically contain 2% by weight of TCC. Concentrations of TCS in this and other personal care and household products are lower, primarily in the range of 0.1 – 0.3% by weight (Sabaliunas et al. 2003). Many antimicrobial consumer products are washed down the drain after use and thus become part of domestic wastewater treated in municipal sewage treatment facilities. Usage of antimicrobial and antibacterial products is steadily increasing in both the United States and worldwide (U.S.E.P.A. 2003), which implies continual release of these compounds into the environment.

While little is known on fate of TCS and TCC in the environment after graywater irrigation, some studies have raised concern over occurrence of these constituents, their potential bioaccumulation, toxicity, and potential to form antibiotic resistant genes (Halden and Paull 2005; U.S. EPA 2009; Higgins et al. 2009, Wu et al. 2009, Birošová et al. 2009, Yazdankkhah et al. 2006). The fate of TCS and TCC in the environment is of interest to environmental scientists and regulatory agencies alike. Stimulus for environmental exposure and risk assessments is provided by various reports on increased microbial resistance following exposure to household biocides (Yazdankhah et al. 2006), ecotoxicity to aquatic organisms (Orvos et al. 2002, Reiss et al. 2002), the potential for generation of toxic biocide degradates in the environment (Latch et al. 2005, Sanchez-Prado et al. 2006), and the possibility of adverse human health effects inferred from work with animal models (Chen et al. 2008). In addition to these toxic effects, both biocides have been shown to bioaccumulate in aquatic species, and TCS also has been detected in human milk (Adolfsson-Erici et al. 2002, Coogan et al. 2007). Furthermore, TCS and TCC have been observed to persist in the environment for extended periods of time, particularly under anaerobic conditions (Ying et al. 2007).

1.11. Limitations of Other Studies

A study conducted by the City of Los Angeles (1992) showed that sodium increased in soil after irrigation with graywater; however, negative effects on plant growth and quality of landscape plants were not observed. Travis et al. (2010) observed accumulation of surfactants up to 680 μg kg^{-1} as anionic surfactants when soil was irrigated with graywater. They concluded that this may alter soil properties such as causing higher water repellency. Wiel-Shafran et al. (2006) reported that irrigation with graywater caused the accumulation of surfactants in soil. They also observed that sodium level of the soil increased and hydraulic conductivity and capillary rise of the soil decreased after graywater irrigation.

While previous researches on the fate of graywater constituents in soils have been valuable, the results are limited. Studies reporting surfactant accumulation in soils irrigated with graywater have been conducted with synthetic graywater, addressed limited soil types, and/or have been conducted under laboratory conditions (Wiel-Shafran et al. 2006; Travis et al. 2010). They also have not studied all the contaminants of concern including surfactants, antimicrobials, B, SAR, nutrients and fecal indicators. In addition, other researchers used the MBAS method for surfactant measurement which is an indirect measurement and is susceptible to interferences and less accurate than LC/MS analysis. Direct methylene blue analysis of extracts derived from sludge, sediment, and soil invariably leads to highly inflated estimates of LAS (Berna et al. 1991).

1.12. Objectives

While graywater reuse for household irrigation is widespread, potential effects to soil quality, groundwater quality, and fate and transport of surfactants have not been adequately assessed. The application of any irrigation water will introduce chemicals to the soil and potentially have short- and long-term effects (Figure 1-10). This potential depends on application rate, chemical concentrations in the water, biodegradation rate of the chemical, sorption, leaching, and plant uptake (Figure 1-10).

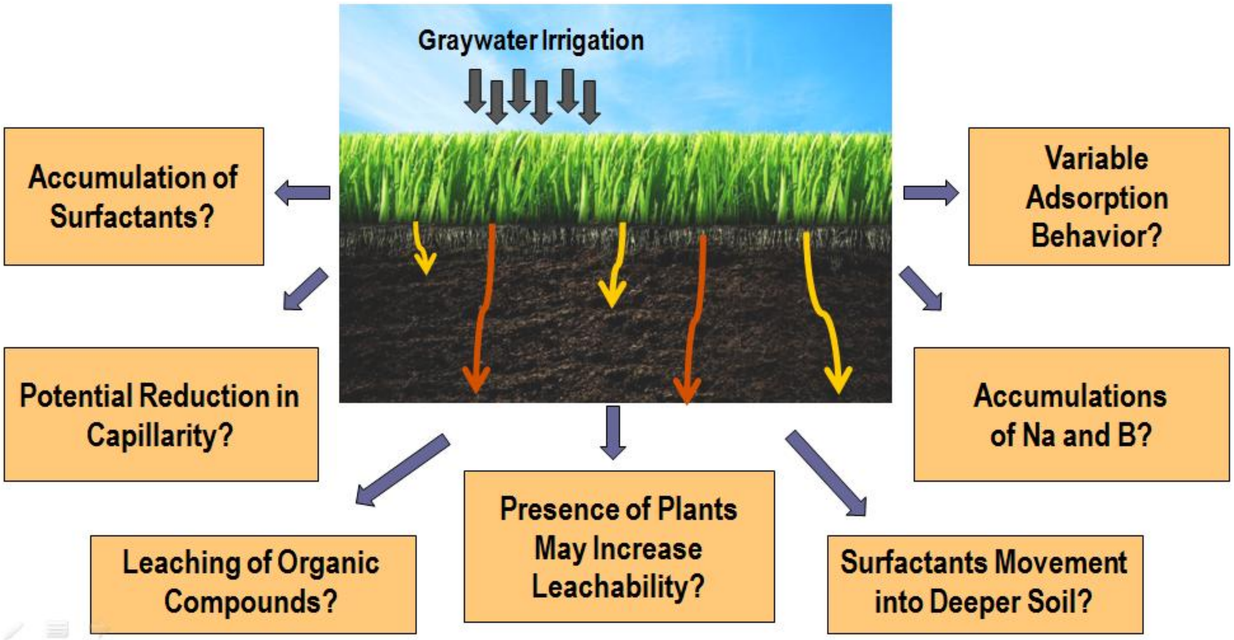


Figure 1-10. Research Questions

Current research has not addressed impacts of graywater chemical constituents on soil quality and leaching potential of these constituents into groundwater. In addition, household graywater has not been adequately characterized. The study proposed herein describes scientific experiments to alleviate these information gaps regarding household graywater irrigation. The aim of this research is to answer if graywater application is safe in terms of its effects on soil quality and fate and transport of graywater constituents. Before Starting the research experiments and based on both our preliminary results and results from other literature, some key hypotheses were derived. Specific objectives (SO) and related hypotheses (H) of this research can be summarized as the following:

SO1. Evaluate the effects of graywater application on accumulation of constituents in soil including surfactants, sodium, nutrients, OM, antimicrobials and B under real conditions in the field

H-1.1. Graywater application causes accumulation of sodium in soil but not to the SAR of 5, the level of concern for plant health, and less than reclaimed wastewater irrigation

H-1.2. Graywater application has no significant impact on boron concentration in soil to the level of concern for plant health

H-1.3. Graywater irrigation increases nitrogen and phosphorus in the soil

H-1.4. Graywater application causes accumulation of surfactants and antimicrobials in soil but less than levels found in bio-solid amended soil

H-1.5. Similar to reclaimed wastewater application, graywater increases the level of organics in soil after long-term application

SO2. Evaluate leaching potential of graywater constituents

H-2.1. After application of graywater for irrigation, surfactants mainly retain in the surface soil and do not leach to deeper soil

H-2.2. Antimicrobials do not leach to deeper soil

H-2.3. Continuous irrigation with graywater causes leaching of nitrogen especially in the form of nitrate

H-2.4. Continuous irrigation with graywater causes leaching of salts

SO3. Evaluate and compare mobility of surfactants in soil columns in the absence of biodegradation

H-3.1. Surfactants within the range of concentrations found in graywater behave similarly in terms of sorption compare to solutions with high concentration of surfactants used in other studies.

H-3.2. Even within the range of surfactant concentrations found in graywater, surfactants have different mobility and leaching rate depends on their characteristics.

H-3.3. There is a correlation between surfactants adsorption and organic content of the soil.

H-3.4. There is a correlation between sorption of surfactants and clay content of the soil.

SO4. Determine the effect of graywater application on soil hydrodynamics including infiltration and hydraulic conductivity

H-4.1. Graywater has lower surface tension which causes reduction in capillary rise and affects movement of water trough soil pores in unsaturated zone

1.13. Research Approach

The research approach was to evaluate accumulation of chemicals, fate and transport of surfactants, and changes in soil properties due to application of graywater for irrigation through several experimental studies (Figure 1-11). Field experiments and greenhouse study provided data on accumulation and leachability of surfactants, salts, and nutrients after short and long-termgraywater application. Column studies will focus on mobility of surfactants in soil column and changes in soil hydrodynamics due to graywater application. In addition, through a combination of greenhouse and column study results, a complete picture of behavior and fate of surfactants and accumulation of salts in soil will be obtained. Furthermore, investigation of soil hydrodynamic properties in greenhouse and column studies will allow better identification of changes in soil properties after graywater application.

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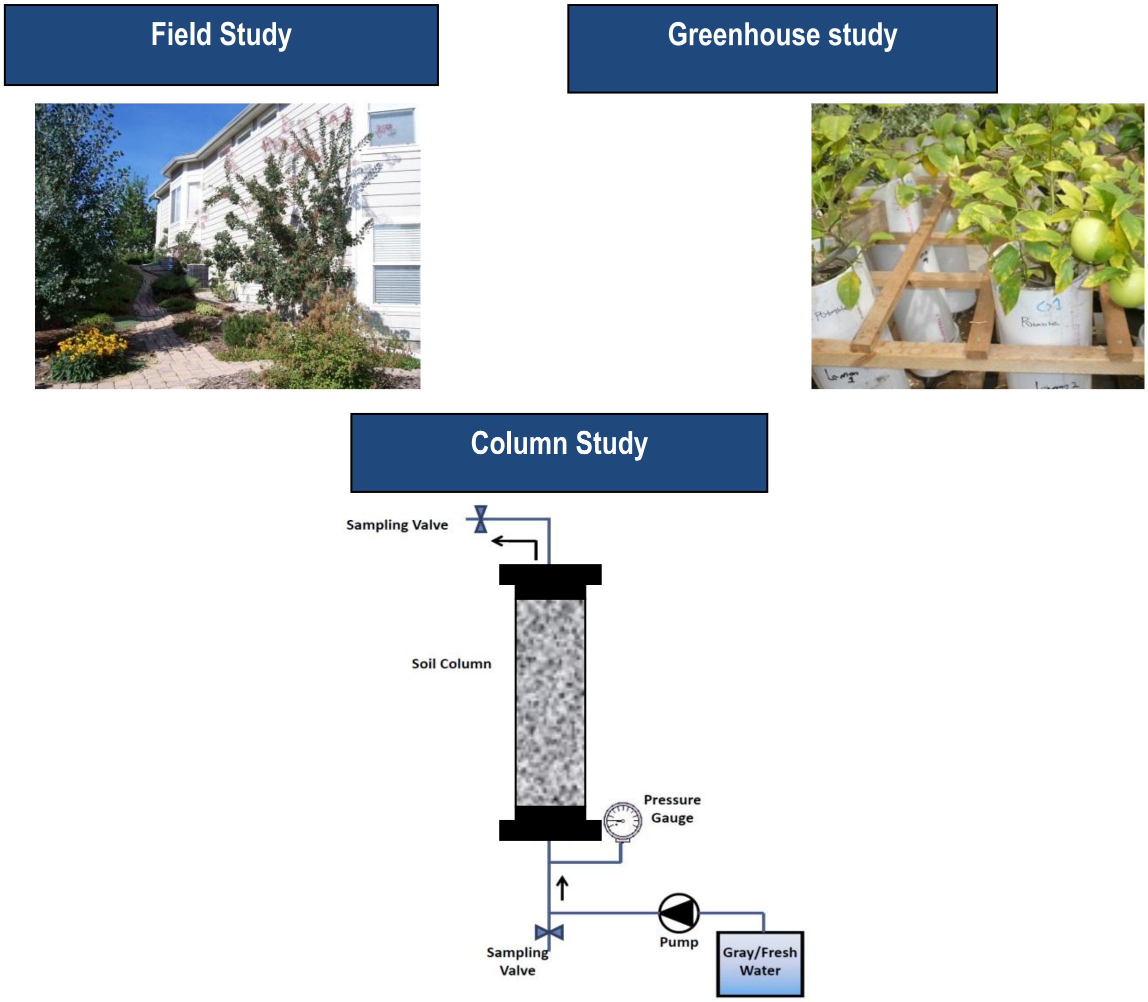

Figure 1-11. Schematic of Research Approach

Based on results obtained from this study two scientific manuscripts have been submitted to peer reviewed journals and two others are under preparation. Chapter 1 contains an introduction to the study, a thorough literature review and objectives of the research. Chapter 2 represents our first manuscript based on our filed data from existing households with graywater irrigation systems for more than 5 years. This manuscript has been accepted for publication at Journal of Water, Soil and Air Pollution. Chapter 3 provides a detailed overview of results obtained from new households. This chapter is under preparation and will be submitted to the Journal of Ecological Engineering. Chapter 4 represents our results obtained from greenhouse study. This chapter has been submitted as a manuscript to the Journal of Science of the Total Environment. Chapter 5 presents the preliminary results we obtained from the column study. The chapter will be submitted to Chemosphere after preparation. Chapter 6 presents the conclusions and suggests future work that can be undertaken as an extension of this study.

1.14. Significance of the project

This is the first study combines the results of graywater application on soil quality obtained from the field (households with long-term history of graywater application and households with short-term graywater application), and from the greenhouse (controlled application of graywater in the greenhouse). In addition, this is the first study evaluating impacts of graywater irrigation in different soil-plant systems in a controlled environment to develop scientifically justified conclusions regarding the leaching potential of graywater constituents. In addition to field and greenhouse studies, a set of column studies were conducted to provide more scientific data on the mobility of surfactants through soil in the absence of biodegradation.

Consequently, results obtained from this study:

- Provides science based data on effects of graywater irrigation to soil quality, which can be applied to make informed decisions on graywater reuse.
- Addresses leaching of graywater chemical constituents through soil and potential for groundwater contamination.
- Provides scientifically sound conclusions as both field studies and controlled studies in a greenhouse were conducted.

Chapter 2

FATE OF GRAYWATER CONSTITUENTS AFTER LONG-TERM APPLICATION FOR LANDSCAPE IRRIGATION[1,2]

2.2. Introduction

Recent concerns over limited water resources in arid/semi-arid regions and overloaded wastewater treatment facilities have increased interest in new water management strategies. One approach that is gaining popularity is reuse of graywater for non-potable uses such as landscape irrigation and toilet flushing. By the most general definition, graywater is domestic wastewater not generated from toilet flushing and this definition is used particularly in Europe and Australia. However, in the U.S., the more common definition of graywater is wastewater that originates from laundry, bathtubs, showers and sinks and does not include wastewater from kitchen sinks, dishwashers and toilets (Eriksson et al. 2002). Graywater comprises about 50% of total wastewater generated within a household (Mayer et al. 1999). In 1999, the Soap and Detergent Association estimated that about 7% of U.S. households were reusing graywater (NPD 1999). This number is likely now even larger as many states have begun to allow graywater reuse over the last several years (ADEQ 2003). The most simple reuse application for graywater is landscape irrigation because simple treatment systems are often applied as compared to more

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[1] Masoud Negahban-Azar performed surfactant and antimicrobial analysis, conducted statistical analysis for all parameters and prepared the manuscript. All phases of the project and manuscript were supervised by Sybil Sharvelle. Mary Stromberger performed the analysis for indicator organisms, interpretation of the result and related discussion. Christopher Olsen performed infiltration tests and samples collections in the field. Larry Roesner provided technical comments.

[2] Accepted for publication at Journal of Soil, Water and Air Pollution.

complex system required for toilet flushing, where human contact with water is likely and disinfection is typically recommended. Although reuse of graywater for landscape irrigation is gaining momentum, studies on its impacts on soil properties are limited (Misra and Sivongxay 2009).

Graywater contains a complex mixture of chemicals used in households. The National Institute of Health (2004) reported that household products contain 2500 chemicals in 5000 different products and many of these compounds will be present in graywater. Graywater quality varies from source to source and also within a source based on both when and where the sample was collected in relation to homeowner water uses (Eriksson et al. 2002). When graywater is reused for irrigation, constituents of concern include pathogens and/or viruses, organic content, nutrients (nitrogen (N) and phosphorus (P)), metals, salts, boron (B), sodium (Na), and personal care product ingredients. Several studies have detected fecal contamination of graywater via the presence of indicator organisms (e.g., Rose et al. 1991, Jeppesen 1996, Casanova et al. 2001, and Ottoson and Stenström, 2003), and thus there is potential for graywater irrigation to increase the number of fecal organisms in soil. In a study of eleven households recycling graywater in Arizona, researchers found higher counts of fecal coliforms in soil irrigated with graywater compared to soils irrigated with freshwater, especially if the households had children or included kitchen wastewater in the graywater (Casanova et al. 2001). A previous study by Pinto et al. (2009) showed no significant differences in total N and P in soils irrigated with graywater compared to soil irrigated with freshwater. A study conducted by the City of Los Angeles (1992) showed that sodium increased in soil after irrigation with graywater; however, negative effects on plant growth and quality of landscape plants were not observed. Boron is another concern because it is toxic to plants when presents in irrigation water at 1.8 mg L^{-1}or more

(Mahler 2009; Blevins and Lukaszewski 1998). However, research to date has not reported effects of graywater irrigation to boron concentration in soil. Research on surfactants, antimicrobials, dyes and enzymes is limited. The most widely used surfactants worldwide include LAS (1 M ton/year), AE (0.5 M ton/year), and AES (0.35 M ton/year), which are found in graywater (Modler et al. 2004). While some researchers have shown potential negative impacts of elevated surfactant concentration to soil (Abu-Zreig 2003; Wiel-Shafran et al. 2006), the fate of surfactants after applied to soil is largely unknown. Some recent studies on graywater indicated accumulation of surfactants up to 680 µg kg^{-1}as anionic surfactants when soils were irrigated with graywater and possible alteration of soil properties (Travis et al. 2010). Studies reporting surfactant accumulation in soils irrigated with graywater have been conducted with synthetic graywater, addressed limited soil types, and/or have been conducted under laboratory conditions (Wiel-Shafran et al. 2006; Travis et al. 2010). Triclosan (TCS) and Triclocarban (TCC) are the two commonly used antimicrobials in household and personal care products (Schweizer 2001; U.S. EPA 2002). While little is known on fate of TCS and TCC in the environment after graywater irrigation, some studies have raised concern over occurrence of these constituents, their potential bioaccumulation, toxicity, and potential to form antibiotic resistant genes (Halden and Paull 2005; U.S. EPA 2009; Higgins et al. 2009, Wu et al. 2009, Birošová et al. 2009, Yazdankkhah et al. 2006).

While previous research on the fate of graywater constituents in soils has been valuable, results are limited because the duration of irrigation before data collection has been less than one year in most cases, synthetic graywater has been applied, and field studies have not been conducted. The objective of this research was to elucidate information on the fate and occurrence of graywater constituents and the long-term impacts on soil quality under actual conditions. Four households where graywater was applied for irrigation for more than five years were included in the study. These households were located in Arizona (AZ), California (CA), Colorado (CO), and Texas (TX). These locations represent substantially different climatic and soil conditions. Quantitative data was collected on the fate of graywater constituents and effects on soil quality. While the number of sites included in this study was limited, the intention of this work was to provide insight into the fate of graywater constituents in real conditions and screen major concerns associated with graywater reuse for irrigation which may require more attention. While sample collection at multiple sites inherently results in an uncontrolled environment (climate, soil type, presence of animal fecal material etc.), data collection at such sites enables conclusions to be drawn based on real, uncontrolled systems rather than simulated systems. Of note is that fecal indicator organisms, surfactants, antimicrobials and B were included in the study, in addition to more commonly studied parameters.

2.3. Materials and Methods

2.3.1. Reference Materials and Reagents

Alkyl ethoxy sulfate (AES) was purchased from Stepan Co (Northfield, IL) in the form of STEOL CS 130, CS 270, and CS 330 respectively. STEOL CS 130, CS 270, and CS330 contain sodium lauryl ether sulfate derived from fatty alcohols that are ethoxylated to an average of 1, 2, and 3 moles respectively. STEPANOL DCFAS-P, which is an alkyl sulfate (AS) with no ethoxylate group, was obtained from Stepan Co (Northfield, IL). NEODOL 25-9®, containing 100% pure polyalcohol ethoxylate (AE), was obtained from Shell Chemical Co, (Houston TX). This product is a mixture of 12 and 13 carbon length alkyl chains with an average of 5 moles ethylene oxide per mole of alcohol ethoxylate. Triclocarban (TCC) and triclosan (TCS) (99% purity) was obtained from Sigma-Aldrich (Milwaukee, WI). Linear Alkylbenzene Sulfonate

(LAS), with carbon chain lengths of 10 to 13, were obtained from Proctor and Gamble (Cincinnati, OH). All solvents (methanol, acetone and acetonitrile) were HPLC grade, purchased from Honeywell Burdick and Jackson.

2.3.2. Experimental Design

The four households included in this study were located in Bisbee, AZ, Escondido, CA, Fort Collins, CO, and Dallas, TX. The graywater systems at these homes varied from very simplistic (no treatment or storage) to more complex and were in operation from 5 years up to 31 years at the time of sampling events (Table 2-1). At each household, soil samples were collected in an area irrigated with graywater as well as a control area with analogous soil and landscaping that was irrigated with freshwater. Samples were assessed for soil physical and chemical properties, indicator organisms, surfactants and antimicrobials. Household graywater quality was not analyzed because household graywater quality has been well characterized (Eriksson et al. 2002) and single samples would not have been representative of the water that has been historically applied to the site given the duration of graywater irrigation at the sites. Reported concentrations for constituents of concern for this study are included in Table 2-2.

Table 2-1. Summary of graywater systems at households sampled in this study.

Location	Duration of Graywater Irrigation (years)	System Description	Irrigation Method	Irrigation Frequency	Date of Sampling
Escondido, CA	10	storage, slow sand filter, pump	Submerged Drip	Daily	Oct. 2008
Fort Collins, CO	5	storage, course filter, pump	Hose Application	Manual application as needed	Oct. 2009 Sep. 2010 Sep. 2011
Dallas, TX	31	no storage, direct connect from washing machine	Hose Application	With operation of washing machine	Sep. 2008 Oct. 2009
Bisbee, AZ	5	no storage	Bucket Application	Manual application as needed	Jun. 2009

2.3.3. Soil Sample Collection

Soil samples were collected with 0.8 inch diameter Zero Contamination sampling tubes (JMC Soil Samplers, Newton, IA), connected to a Backsaver Handle (JMC Soil Samplers, Newton, IA). Sampling tubes were lined with a removable PETG copolyester liner to prevent contamination with surrounding soil as the soil sample was pulled up to the surface. A new sampling tube was utilized for each sample collection. Samples were collected as close as possible to the base of a plant that was irrigated with either graywater or freshwater. Three individual soil samples were collected at each of three depth increments (0-15 cm, 15-30 cm, and 30-100 cm) in CO and TX. Depth sampling was not feasible at households in either CA or AZ, due to the shallow depth to bedrock. In AZ, it was observed that applied water primarily flowed laterally rather than vertically through the soil. Therefore, samples were taken at a depth of 0-15 cm within a 0-60 cm, 60-90 cm and 90-120 cm radius of a saltbush irrigated with graywater. After sample collection, removable liners were sealed with vinyl caps, placed on ice in a cooler, and shipped overnight to Colorado State University for analyses. Triplicate samples collected at each depth or location were homogenized in the laboratory manually and treated as one sample. 1 sampling event was conducted at each CA and AZ and two sampling events were conducted in TX and CO (Table 2-1). Sampling events occurred near the end of the dry season in each location, when accumulation of graywater constituents in soil would have been highest.

Table 2-2. Household graywater quality

Source	pH	Chemical Oxygen Demand (mg L^{-1})	Total Nitrogen (mg L^{-1})	Total Phosphorus (mg L^{-1})	Fecal Coliform (per 100 mL)	Sodium Absorbance Ratio	Anionic Surfactants (mg L^{-1})	Reference
Shower and laundry	6.7 - 7.6	278 - 435	-	0.24- 1.2	4.7×10^4 – 8.3×10^5	4.2 – 5.8	-	Finely et al. (2009)
Domestic (unspecified)	8.1 ± 0.1	-	19 ± 1.6	31 ± 6	9.0×10^5	5.9	34 ± 8.2	Wiel-Shafran et al. (2006)
Bath, dish washing and laundry	6.3 - 7.0	702 – 984	25.0 – 45.2	1.72 ± 27	9.0×10^4 – 1.0×10^8	-	4.7 – 15.6	Gross et al (2007)
Bath and laundry	6.4 - 10.0	-	-	0.06 - 42	1.1×10^2 – 3.3×10^3	-	-	Christova-Boal et al. (1996)
Bath	7.3 ± 0.1	435 ± 130	7.2 ± 1.8	2.8 ± 1.3	-	-	4.1 ± 0.6	Travis et al (2008)

2.3.4. Infiltration Tests

Single ring infiltrometer tests were conducted at each household to estimate the infiltration capacity of the soils. For the first several sampling events (conducted in 2008), only 1 infiltration test was performed. However, the number of tests was increased to at least two replicate tests in each of the graywater and freshwater-irrigated areas for future tests. Mulch or ground cover was removed and a 12 inch corrugated pipe was placed on end and rotated with vertical pressure until it penetrated the exposed soil to a depth of 1 ½" to 2". The pipe was then filled with 6 to eight inches of water, and the rate of water level decrease was measured. This provided an estimation of surface soil infiltration rate, which is of interest since graywater is often applied to the soil surface. The purpose of the infiltration tests was to evaluate differences in areas irrigated with graywater compared to those irrigated with freshwater. Comparisons cannot be made between samples collected at different sites or even at the same site at a different time, where soil moisture content and other parameters may have been very different.

2.3.5. Analytical Methods

2.3.5.1. Routine Soil Analyses

Soil texture (particle size) was determined on each sample using the hydrometer method described by Gee and Bauder (1986). Soil samples from the surface (0-15 cm depth, where graywater was expected to have the greatest impacts) were analyzed for pH, electrical conductivity (EC), organic matter, and cations ions including Mg^{2+}, Ca^{2+} and Na^{+} for sodium adsorption ratio (SAR) as calculated by equation 2-1 where concentration is expressed as meq L^{-1}.

(Equation 2-1)

$$SAR = \frac{[Na^{+}]}{\sqrt{\frac{[Ca^{2+}] + [Mg^{2+}]}{2}}}$$

Soil pH was determined by the saturated paste method (Method 21a of USDA Handbook No. 60, 1954) and soil EC was determined by the saturated paste method of Rhoades (1996). Organic matter (OM) was determined by the modified Walkley-Black method described by Nelson and Sommers (1996). Concentrations of Mg^{2+}, Ca^{2+} and Na^{+} were analyzed on an inductively coupled plasma-atomic emission (ICP) spectrophotometer (Thermo Jarrell Ash Corp., Franklin, MA) from a saturated paste extract as described by Sumner and Miller (1996) for SAR. Boron concentration was determined by the hot water extraction method, followed by ICP analysis (Keren 1996).

2.3.5.2.Surfactants

LAS, AES, and AE were analyzed in soil samples. The soil extraction method described by Dyer et al. (2006) was modified for recovery of surfactants from the soil samples. Modifications included using 30 g of soil and changing the shaking, sonication and centrifugation to 20 min (1 min manual plus 19 min mechanical), 10 min, and 10 min respectively. In addition, instead of acetonitrile and methanol/ethyl acetate/water, only methanol was used for the extraction. Soil samples were dried and weighed after extraction and concentrations in soil samples are reported per mass of dry soil. Soil moisture for all samples was within the range of 2 to 5%. Experiments were conducted to determine the recovery rate of each surfactant in soil. Soils (sandy loam, silt clay, and clay loam) with no surfactants were spiked with surfactant (0.5-2.0 mg kg-1). Average rates of recovery were 85±14, 86±15, and 91±11 for LAS, AES and AE respectively for the various soil types.

An Agilent 1200 (Agilent Technologies, Santa Clara CA) high-throughput HPLC system coupled with an Agilent 6220 Accurate Mass Time of Flight mass spectrometer (Agilent Technologies, Santa Clara, CA) was used for determination of LAS, AS/AES, and AE. The analytical method was developed based on other research and methods by SDA for surfactants measurement (McAvoy et al. 1993, van Compernolle et al. 2006, Sanderson et al. 2006 and Sanderson et al. 2006).The data were controlled and processed using MassHunter Workstation software (Agilent Technologies, Palo Alto CA) and is described in Table 2-3.LAS, AS and AES were quantified by monitoring 297 + (Δ14),265 and 309 + (Δ44) m/z respectively. In addition, AE was quantified by monitoring of two ions of 614 and 658 m/z which had highest detectable peaks as surrogates.

Two procedural blanks were run for each set of samples to evaluate potential contamination from sample preparative steps. If surfactants were detected in blanks, extractions were repeated. Two blanks and two samples with known concentration were run for each set of fifteen samples to evaluate potential contamination for general quality control. Detection limits for LAS, AES and AE were 5 µgL^{-1}, 5 µgL^{-1}, and 3 µg L^{-1} in liquid phase and 0.5 µg kg^{-1}, 0.5 µg kg^{-1} and 0.3 µg kg^{-1} respectively in soil samples (based on specific soil extraction protocol previously described).

2.3.5.3.Antimicrobials

For extraction of antimicrobials (TCS and TCC), 10 g of soil sample was transferred to a 50 mL conical centrifuge tube. A volume of 25 mL of methanol/acetone (50/50 volume) was added, followed by hand shaking for 5 minutes, automated shaking for 30 min, and sonication for an additional 10 minutes. The sample was centrifuged (2500 rpm for 10 min) and the clear solvent was decanted to a separate conical centrifuge tube. The methanol/acetone extraction was repeated (once) with the same soil sample and additional methanol was added to the first extract (giving a total methanol/acetone volume of approximately 50 mL). A gentle stream of nitrogen gas was used to evaporate the methanol/acetone extract. A volume of 1 mL methanol was added to the tubes and tubes were centrifuged for an additional 5 minutes to ensure that all TCS/TCC was captured in the liquid solution. After centrifugation, samples were filtered (0.45 µm sterile cellulose acetate membrane centrifuge filter) and placed in 2 mL vials for LC/MS analysis. When samples were not processed directly after extraction, the samples were stored in a freezer (-6 ^{o}C). Antimicrobial free soil (sandy loam) was spiked with TCS and TCC (50-100 µg kg^{-1}), and recovery rates were determined to be 91%±6% and 88%±9% respectively (n = 3).

The instrumentation applied for quantification of surfactants (LC-TOF MS) was also used for detection TCC and TCS (Table 2-3). TCS and TCC were quantified by the monitoring of 285 and 314 m/z respectively. Given the extraction method, the detection limit in soil samples was 0.4 µg kg^{-1} and 0.2 µg kg^{-1} respectively. Quality control measures as described for surfactant analysis were applied for quantification of TCS and TCC.

Table 2-3. Summary of analytical procedures

Compound	Ionization Mode	Column	Column Temperature (^oC)	Injection Volume (µL)	Flow Rate (mL/min)	Solvents	Gradients Method
LAS C_{10}-C_{13}, AS (C_{12}) and AES (C_{12} EO_{1-3})	negative	XTerra® MS C18 column (2.5 µm, 50×2.10 mm), Waters Corp, (Milford, MA)	40	10	0.32	1- water + 20 mM ammonium acetate 2- acetonitrile + 20 mM ammonium acetate	1. 60% water and 40% acetonitrile 2. linear increase to 65% acetonitrile within 20 min 3. linear increase to 80% acetonitrile within 10 min 4. constant 80% acetonitrile for 5 min 5. linear decrease to initial condition within 1 min 6. postrun at initial condition for 10 min
AE	positive	Allure C18 (5 µm, 150×2.1 mm), Restek, (Bellefonte, PA)	40	10	0.32	1- water + 5 mM ammonium acetate 2- methanol + 5 mM ammonium acetate	1. 60% methanol and 40% of water for 10 min 2. linear increase to 95% methanol within 5 min 3. constant 95% methanol for 8 min 4. linear decrease to initial condition within 1 min 5. postrun at initial condition for 10 min
TCS and TCC	negative	Allure C18 (5 µm, 150×2.1 mm), Restek, (Bellefonte, PA)	35	5	0.32	1- water 2- methanol	1. 40% water, 60% methanol 2. linear increase to 100% methanol within 5 min 3. constant 100% methanol for 11 min 4. linear decrease to initial conditions within 2 min 5. postrun at initial condition for 10 min

2.3.5.4.Fecal Indicator Organisms in Soil

Within 24 hours of sampling, individual soil cores were composited by depth and irrigation water treatment. Samples were homogenized by hand and a subsample of each soil was shipped overnight on ice to EMLab PandK Laboratories in San Bruno, CA for most probably number (MPN) enumeration of *Clostridium perfringens*. The remaining soil was analyzed for total coliforms, *E. coli,* and enterococci using the IDEXX Quanti-Tray® enumeration procedure with Colilert® reagent for total coliforms and *E. coli* and Enterolert™ reagent for enterococci. Soil subsamples (20 g) were added to sterile phosphate buffered saline and blended on high speed for 1 minute in sterile Waring blender cup to achieve a 1:10 dilution. Soil suspensions were serially diluted in 100 mL sterile glass bottles and added to Quanti-Trays® after the appropriate reagent was added. Sealed trays were incubated for 24 h at 35°C±0.5°C (total coliforms, *E. coli*) or 41°C±0.5°C (enterococci), after which the MPN of total coliforms, *E. coli*, and enterococci were determined.

2.3.5.5.Soil Dehydrogenase Activity

Dehydrogenase is an intracellular enzyme involved in aerobic metabolic processes, and its activity in soil is often measured to indicate the effects of organic contaminants and wastes on soil biological quality (Margesin et al. 2000, Gil-Sotres et al. 2005). Here, dehydrogenase activity was measured to indicate the response of microbial metabolic activity to graywater irrigation. Triplicate soil subsamples (1 g) were analyzed for dehydrogenase activity following the method of Trevors (1984). Subsamples were incubated at 25°C for 24 h in the presence of 2(ρ-iodophenyl)-3-(ρ-nitrophenyl)-5-phenyl tetrazolium chloride (INT), and the product formed (iodonitrotetrazoliumformazan; INF) was quantified spectrophotometrically at 490 nm, against an INF standard curve.

2.3.6. Statistical Analysis

Statistical packages SPSS 5.0 for WINDOWS (SPSS Inc., Chicago, IL) and Microsoft Excel for Windows (Microsoft Corporation, Redmond, WA) were used for statistical analysis. For all sampling events combined, the significance of irrigation treatment (graywater or freshwater) effects on soil surfactant concentration, SAR, and E. coli was determined at the 95% confidence interval using a 2-way analysis of variance (ANOVA). The 2-way ANOVA was applied with irrigation type and sampling event as the two factors to address variability among sampling locations and sampling time. Of note is data collected from CA sampling location was not included in statistical analysis. Data collected from this site was difficult to interpret and freshwater irrigated areas appeared to be exposed to graywater as a result of highly sloped terrain and shallow depth to bedrock. Data collected from the USGS web soil survey indicates that the land on this property is characterized by a slope of more than 15% in all locations.

In addition to the 2-way ANOVA, ordinary least squares regression (Washington et al. 2003) analysis was conducted to determine what parameters (i.e. soil quality or irrigation type) impacted surfactant concentration, SAR, and numbers of indicator organisms (*E. coli* and Enteroccoci) in soil. For this approach, a regression model was developed between p independent variables (x_j; $j=1,\ldots, p$) and the desired predicted output ($\hat{Y}$) as follows :

(Equation 3-2)

$$\widehat{Y_i} = \beta_0 + \sum_{j=1}^{p} \beta_j x_{j,n}$$

Where β_0 represents the y-intercept, β_j's are estimated coefficients and n is the total number of observations. The magnitude of the coefficient β_j depicts the effect for variable x_j with respect to the variation in dependent variable $\hat{Y}$ for observation i. For this particular study, the dependent variables were measured surfactant concentration, SAR, and number of indicator organisms while the independent variables were irrigation type, percent clay and OM. A multivariate linear regression equation was developed between independent variables and measured parameters of interest using ordinary least squares, where the sum of squared errors is minimized in XLSTAT software (Addinsoft, New York, NY). The ratio of data variance explained by the model to total data variance (R^2) is reported.

2.4. Results and Discussion

2.4.1. Soil physical and chemical properties

General soil quality parameters were measured for all samples (Table 2-4). Heterogeneity in soil texture existed between the graywater-irrigated and control areas at two of the sampling locations (CA and TX). However, all soil samples collected from CA and TX were loam variants and therefore it is not expected that soil texture would have more impact on soil quality parameter studied here than graywater application. Nonetheless, differences in soil texture were considered for interpretation of soil quality data and are discussed below where necessary. In AZ, graywater-irrigated surface soil contained 70% less OM compared to soil receiving freshwater (Table 2-4). The same trend was observed in CA where OM content was 30% less in the graywater-irrigated surface soil than in freshwater-irrigated soil and CO where OM was 38%, 71% and 26% less in the graywater-irrigated area compared to the freshwater-irrigated for 2009, 2010 and 2011 sampling events respectively. This result was somewhat unexpected given the organic concentrations typically observed in graywater (Table 2-2). Consistent with this result, Tarchouna et al.(2010) observed lower values of organic carbon in soil irrigated with reclaimed wastewater in comparison to soil irrigated with freshwater, and attributed this trend to enhanced microbial activity resulting from more available nutrients and dissolved organic carbon. Graywater also serves as a contribution of N and P (Table 2-2) when applied for irrigation. In addition, results from a study conducted by Lanfax Laboratories, Australia (2009) showed that irrigating soil with laundry water rendered soil organic matter more soluble, increasing leaching of organics from the soil. Of note is that this trend was not observed in TX where organic matter in surface soil irrigated with graywater was higher than freshwater-irrigated soil. Soil pH was similar between fresh and graywater-irrigated soils at each household.

Boron varied among sampling locations, and even between years at the TX and CO households (Table 2-4). Boron concentrations in TX were 45-50% greater in graywater-irrigated soil than in freshwater-irrigated soil despite the large inter-annual variability. Otherwise, extractable B levels were similar between the two soil areas (CA and CO 2009 and 2011), or slightly lower in the graywater-irrigated soil (AZ and CO 2010). Results indicate that irrigating with graywater can increase B concentration in soil. Hot water-extractable B is a good indicator of plant available B at the time of sampling, and soil concentrations of 5-8 mg kg^{-1}or higher is considered toxic to many plant types (Nable et al. 1997). Extractable B concentration in the TX graywater-irrigated soil in 2009 was high enough to warrant concern regarding B toxicity to plants.

Table 2-4. Physicochemical properties of soil (0-15 cm depth) receiving freshwater or graywater at households with an existing graywater installation system.

Location	**Irrigation**	**Sand** (%)	**Silt** (%)	**Clay** (%)	**Texture**	**pH**	**Organic Matter** (%)	**Boron** (mg kg^{-1})
Bisbee, AZ	Freshwater	78	12	10	Sandy loam	7.9	5.4	7.6
	Graywater	58	23	19	Sandy loam	7.6	1.5	6.8
Escondido, CA	Freshwater	49	30	21	Loam	7.2	4.6	1.1
	Graywater	60	22	18	Sandy loam	7.4	3.2	1.1
Fort Collins, CO	Freshwater[2]	36	28	36	Clay loam	7.5	2.9	2.0
	Graywater[2]	39	27	34	Clay loam	7.6	1.8	2.0
	Freshwater[3]	40	28	32	Clay loam	7.3	5.2	6.2
	Graywater[3]	32	29	39	Clay loam	7.7	1.5	4.7
	Freshwater[4]	22	26	52	Clay	7.2	2.3	0.7
	Graywater[4]	16	28	56	Clay	7.8	1.7	0.7
Dallas, TX	Freshwater[1]	43	26	31	Clay loam	7.5	2.8	0.6
	Graywater[1]	47	24	29	Sandy clay loam	7.5	7.3	0.9
	Freshwater[2]	50	32	18	Loam	7.4	2.5	6.1
	Graywater[2]	47	27	26	Sandy clay loam	7.4	4.5	8.8

[1] 2008
[2] 2009
[3] 2010
[4] 2011

Sodium accumulation has been a problem for reclaimed water irrigation and is also a concern for graywater irrigation (Qian and Mecham 2005). Graywater SAR is expected to range from 4.2-5.9 based on values from the literature (Table 2-2). SAR and EC varied among sampling locations and irrigation treatment (Figure 2-1). In TX, SAR levels were greater by nearly 100% or more in graywater-irrigated soil than in freshwater-irrigated soil, for both years. SAR was slightly higher in graywater-irrigated soil than in freshwater-irrigated soil at the CA household. SAR levels were not notably different at the CO sampling location in the graywater-irrigated area compared to the freshwater-irrigated area (Figure 2-1) where SAR was slightly higher in the graywater-irrigated area in 2009 and 2011 while being slightly lower in the graywater-irrigated area in 2010 compared to the freshwater-irrigated area. In AZ, Na concentrations were below the limits of detection, resulting in SARs near zero. Soil EC was generally similar between the two treatment areas at each household, except in CA where EC was lower in soil receiving graywater instead of freshwater. Results from the ANOVA indicated a significant impact of graywater irrigation on soil SAR ($P \leq 0.05$), with an average SAR of 0.8±0.6 in graywater irrigated soils and 0.6±0.4 in freshwater irrigated soils. The multivariate regression model also showed a significant effect of graywater irrigation on SAR, with soil organic matter also having an effect (Table 2-5). Other researchers have reported that short-term graywater application led to an increase in soil SAR, but not to a detrimental level (Travis et al. 2010; City of Los Angeles, 1992). For example, Travis et al. (2010) reported an increase in SAR from 0.87 to 1.77 after short-term application of raw graywater for irrigation. The structure of some soils can be adversely affected by Na when SAR levels are more than 5 (Mace and Amrhein 2001), and SAR was below 5 in all soil samples collected for this study. Overall, SAR and EC were low in all soil samples, regardless of irrigation type.

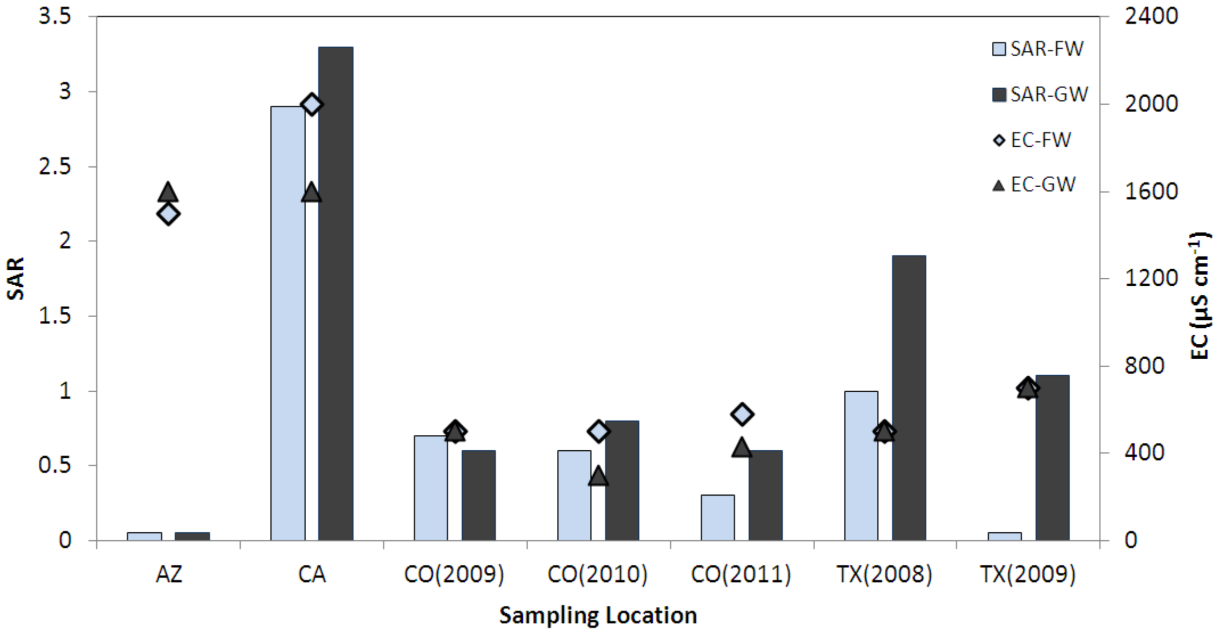

Figure 2-1. SAR and EC in surface soil samples (0-15 cm)

Table 2-5. Results of multivariate regression model for surfactants, SAR, *E. coli* and Enterococci.

Dependent Variable	**Explanatory Variables (*β*)**			**Intercept (β_o)**	**R^2**
	Clay (%)	**OM (%)**	**Irrigation Water [a]**		
Surfactants (µg kg^{-1})	-3.5	0 [b]	855	150	0.66
SAR (-)	0 [b]	0.13	0.65	0 [b]	0.53
E. coli (MPN g^{-1})	0 [b]	55	0 [b]	0 [b]	0.37
Enterococci (MPN g^{-1})	-570	5400	0 [b]	0 [b]	0.49

a: Qualitative Parameter (1: graywater, 0:freshwater)

b: This parameter is not included in the model (t-statistic <1)

2.1.1. Infiltration Capacity of Soils

One concern with graywater irrigation is decreased ability of water to infiltrate into soil over time, which may result in pooling of graywater in sites irrigated with graywater. Because the purpose of infiltration tests was to compare among the graywater and freshwater-irrigated locations at a single sampling event, the difference in infiltration rate between the graywater and freshwater-irrigated areas are reported (Table 2-6). Among households included in this study, infiltration rates were higher in graywater-irrigated areas than freshwater-irrigated areas at CO and TX (Table 2-6). However, at the AZ and CA sampling sites, freshwater-irrigated areas had higher infiltration rates than graywater-irrigated areas (Table 2-6).Of note is that at the AZ sampling site, the clay content was notably higher in the graywater-irrigated area (19%; Table 2-4) compared to the freshwater-irrigated area (10% clay content; Table 2-4). Soil composition was generally more similar at other test locations in the graywater and freshwater-irrigated areas (Table 2-4). Data from the CA household is difficult to interpret due to high bedrock at the site and highly sloped ground surface. Soil infiltration rate may be influenced by irrigation water quality as well as soil texture. For example, an increase in soil sodicity can reduce water infiltration rates into soil (Oster and Shcroer 1979). Borselli et al (2001) reported that a silty clay soil was more affected by the sodium content of irrigation water than a silt loam soil with respect to infiltration. The onset of clay swelling and dispersion is dependent on not only the sodium content and SAR of the soil but also on the overall salt content and hence ionic strength of the soil solution. Among our sampling locations, CO samples had the highest clay content (34-56%; Table 4), however a higher infiltration rate was observed in graywater-irrigated soil than freshwater-irrigated soil at both the 2009 and 2010 sampling events. Graywater irrigation does not appear to have impacted sodium content to result in swelling of clay and decrease the infiltration at this sampling location. While some researchers have inferred that long-term irrigation with graywater may result in decreased soil infiltration rates (Abu-Zreig et al. 2003;

Travis et al. 2010), a consistent decrease of infiltration rate was not observed in graywater irrigated soil sampled for this study.

Table 2-6. Difference in the infiltration rate in the graywater and freshwater irrigated areas at each sampling event (a positive sign indicates that infiltration was higher in the graywater-irrigated area and a negative sign indicates that graywater was lower in the graywater-irrigated area compared to the freshwater irrigated).

Location	Year	Infiltration Rate Difference (in h^{-1})
AZ	2009	-17.0 ± 14.7 [a]
CA	2008	-10.6
CO	2009	0.2
	2010	13.0 ± 19.9[b]
	2011	6.1 ± 11.4[b]
TX	2008	30.0
	2009	10.1 ± 14.7[b]

[a] n = 2

[b] n = 3

2.4.2. Surfactants

A large component of the organic content in graywater is surfactants and total anionic surfactants have been found to range from 4-34 mg L^{-1} (Table 2). Measured concentration of LAS (C_{10}-C_{13}), AES (C_{12} EO_{0-3}), and AE (C_{12} EO_{0-9}) at each household were summed to determine total surfactant concentration in surface soil samples (0 - 15 cm) collected from households with existing systems (Figure 2-2). In surface soil samples, the averaged total surfactant (over all sites) was 0.139±0.880 and 0.029±0.025 mg kg^{-1}in graywater-irrigated and freshwater-irrigated soil respectively. With the exception of the CA household, graywater-irrigated areas contained higher surfactant concentration than freshwater-irrigated soil samples (Figure 2-2). The CA site was highly sloped and migration of graywater into areas not irrigated by graywater was a possibility. Results from the 2-way ANOVA (where data from the CA household was omitted) indicated that graywater irrigation significantly impacted surfactant concentration in surface soil ($P \leq 0.05$). Multivariate regression analysis showed that in addition to irrigation treatment impacting surfactant concentration, clay content was negatively correlated to soil surfactant concentration (Table 5). While the relative ratios of AE:AES:LAS are variable among all locations, AS/AES was the dominant surfactant detected in soil collected from graywater-irrigated and freshwater-irrigated areas at all locations sampled except for CO in 2011 (Figure 2-2).

Surfactant concentrations measured in our study were lower, but comparable to those reported in another study by Travis et al. (2010) where total surfactant was reported to be 0.68±0.39, 0.15±0.06 and 0.53±0.14 mg kg^{-1}in sand, loam and loess irrigated with raw graywater respectively. Meanwhile, Wiel-Shafran et al. (Shafran et al. 2005 and Wiel-Shafran et al. 2006) in two studies, reported up to 60 mg kg^{-1} and 30±7.2 mg kg^{-1} accumulation of anionic surfactants in soil receiving graywater using the MBAS method, much higher that than the maximum total anionic surfactant concentration observed in this study (0.15 mg kg^{-1}). However, of note is that Wiel-Shafran (2006) also reported surfactants in control areas irrigated with freshwater between 5 and 6 mg kg^{-1}. These values seem excessively high, given that biosolids amended soil has been found to contain 16 and 53 mg kg^{-1}of LAS immediately after biosolids application containing 7000 and 30,200 mg kg^{-1} respectively and were less than 0.3 mg kg^{-1} within 90 days (Berna et al.1989). It should be also considered that several compounds can be methylene blue-reactive, interfere with the results, and overestimate the surfactant concentration. Direct methylene blue analysis of extracts derived from sludge, sediment, and soil invariably leads to highly inflated estimates of LAS (Berna and Moreno1991).

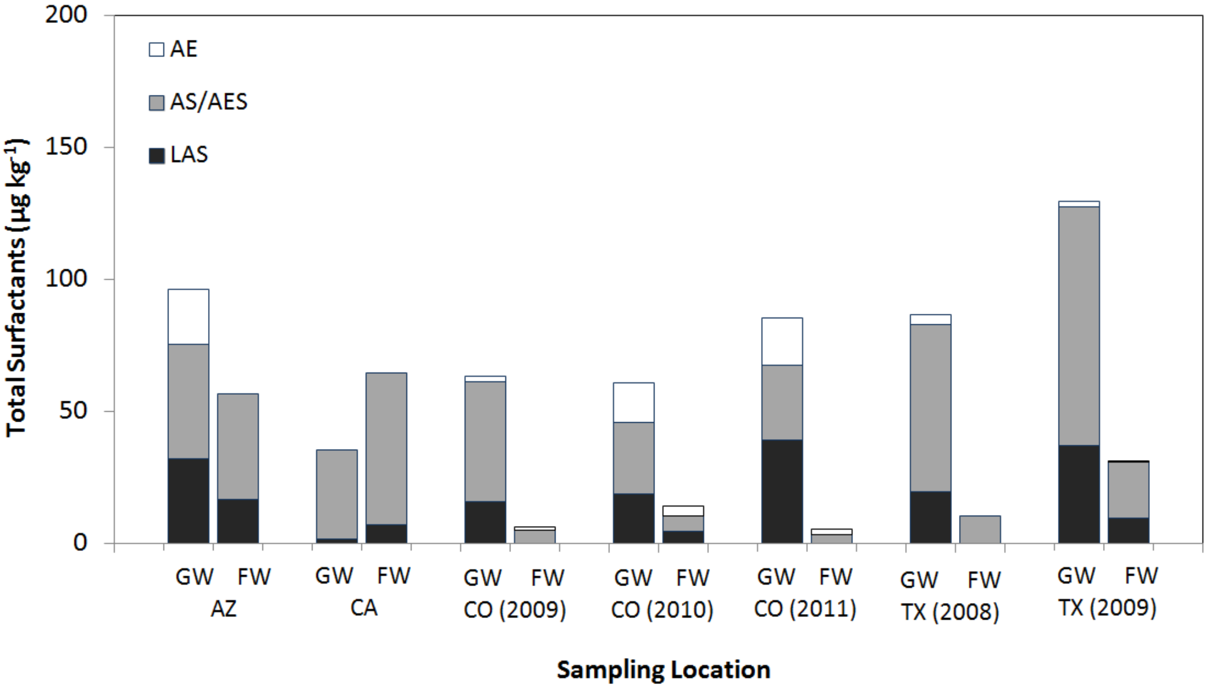

Figure 2-2. Total Surfactants Concentration (including LAS, AES, AS, and AE) in Surface Soil Samples (0-15 cm) (gw: graywater-irrigated, fw: freshwater-irrigated)

Surfactant concentration in graywater-irrigated soil can be compared to biosolids amended soil. LAS concentration in soil samples amended with biosolids ranged from 0.19 to 5.0 mgkg^{-1} within 76 days to 12 months after application (Marcomini et al. 1989 and Figge and Schoberl 1989). For the four homes included in our study, the average detected concentration of LAS in graywater-irrigated surface soil was 0.026±0.020 mg kg^{-1}, much lower than concentrations found in biosolids amended soil. Of consideration is surfactant toxicity. Several studies have been conducted on risk assessment of LAS, AS/AES and AE mainly in aquatic environment and river sediments (DK-EPA 2001), however such data is not available for soil inhabiting organisms. It is not possible to relate soil surfactant concentration to toxicity for soil inhabiting organisms based on toxicity data currently available.

Depth soil samples were collected from households in TX and CO (Figure 2-3). Total surfactant concentration decreased with soil depth in CO samples from all years. However, at the

TX household, total surfactants concentration increased substantially with soil depth in the graywater-irrigated area in 2008 (Figure 2-3). This may be a result of the very high infiltration rate determined for this soil (Table 5) and/or potential anaerobic conditions in the deeper soil, resulting in slower biodegradation. In fact, soil samples below 30 cm were found to be saturated with groundwater at the 2009 sampling event. It is expected that surfactants will adsorb to soil (Ying 2006 and Boluda-Botella et al. 2010). However, the sorption of LAS was reversible according to data reported by Boluda-Botella et al. (2010). As a result, anionic surfactants may reach deeper soil if sufficient water is applied to the soil. This may explain the occurrence of surfactants in the deeper soil samples observed in TX. According to Ying (2006), some surfactants (e.g. LAS) are persistent to anaerobic biodegradation and the anaerobic conditions in deep soil may favor the existence of surfactants in the depth samples after they penetrate to the deeper soil (Ying 2006). This is consistent with our results which show increasing concentrations of LAS in all depth samples (30-100 cm), even when total surfactant decreased (Figure 3). Interestingly, an increasing trend of surfactant concentration with soil depth was not observed in samples collected at the 2009 TX sampling event (Figure 2-3). While surfactant concentration was lower in the depth samples (30-100 cm) compared to surface samples (0-15 cm), notable concentrations of surfactant were detected in the depth sample at the TX site in 2009 (82.4 mg kg^{-1}).

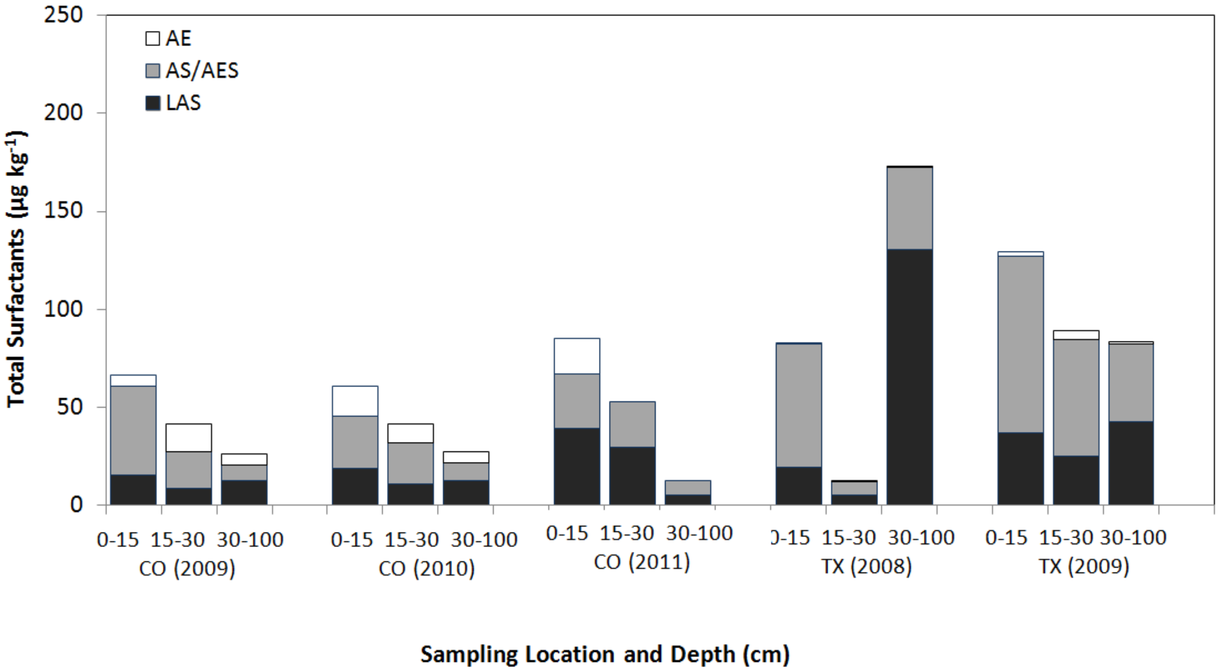

Figure 2-3. Total Surfactant Concentration in Graywater-irrigated Soil at Different Depths

As described in the methods section, soil samples were collected at different distances from the root zone in AZ instead of depth samples. In the graywater-irrigated soil samples, LAS and AES/AS concentration first increased within the distance from the root zone and then decreased (Figure 2-4). Because the plant was irrigated exactly within the root zone, one may expect the highest surfactant concentration to be observed in the area closest to the plant base. However, it is possible that increased microbial activity within the root zone resulted in lower surfactant concentration than observed in samples collected with a 60-90 cm radius of the plant base. Results from microbial data showed that dehydrogenase enzyme activity were 3790, 2138, and 2600 µg kg^{-1} hr^{-1} in 0-60, 60-90 and 90-120 cm samples respectively, indicating the highest microbial activity near to the root zone, consistent with lowest observed concentration of LAS and AES/AS. Of note is AE was only detected in the 0-60 cm sample.

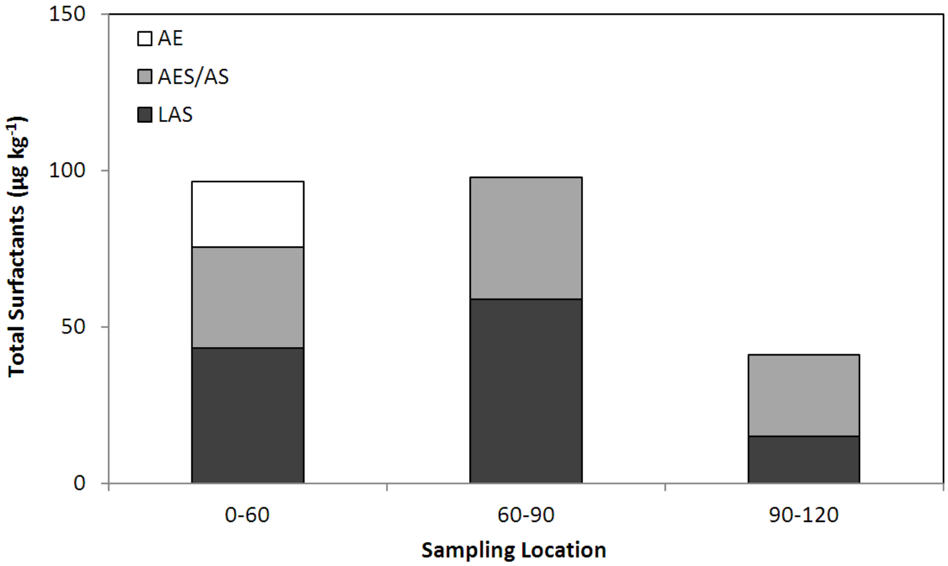

Figure 2-4. Total Surfactants Concentration in Soil Samples Collected from the Arizona Household (0-2', 2'-3' and 3'-4' radius of a saltbush irrigated with graywater for more than five years).

2.4.3. Antimicrobials

Antimicrobial concentrations in surface soil samples (0-15 cm) irrigated with graywater were detected at five of the seven sampling events as 3.8 and 6.3 µg kg^{-1} TCS and TCC in AZ, 3.5 and 9.1 µg kg^{-1} TCS and TCC in CO (2009), 6.3 and 8.4 µg kg^{-1} TCS and TCC in CO (2010), 5.3 and 2.5 µg kg^{-1} TCS and TCC in CO (2011) and 2.8 µg kg^{-1} TCC in TX (2009). TCS and TCC concentrations were below detection limits in surface samples collected from the CA household and TX household in 2008, in all depth samples and in all surface samples irrigated with freshwater. Cha and Cupples (2009) have reported TCS and TCC concentrations in biosolids to be 90-7,060 and 4,890-9,280 µg kg^{-1} respectively, and concentrations of 0.05-1.02 µg kg^{-1} TCS and 1.20-65.10 µg kg^{-1} TCC have been detected in previously amended soil samples with an estimated application rate of 3.25 dry tons per acre. In surface soil receiving graywater for irrigation, the maximum observed concentration of TCS was 3.8 µg kg^{-1}and TCC was 9.1 µg kg^{-}

[1]. TCS was higher in graywater-irrigated soils tested here than observed by Cha and Cupples (2009) in biosolids amended soil while TCC concentration was lower. The concentration of TCS and TCC were notable in those samples where they were detected. A concern associated with high concentration of antimicrobials in soil would be decreased microbial activity. However, dehydrogenase enzyme activity was not consistently lower in soil samples collected from graywater-irrigated areas compared to control areas when antimicrobials were detected in the graywater-irrigated area (data not shown). Further investigation is warranted to determine the effect of graywater irrigation on antimicrobial concentration in soil and the impact this may have to soil microbiology and the potential formation of antibiotic resistant genes.

2.4.4. Fecal indicator organisms

In this study, numbers of *E. coli* were 1 g^{-1} soil or below levels of detection in soil samples collected from AZ, CA, and CO, whereas relatively high numbers were detected in TX soil (Table 2-7). Of note is that chickens, dogs, and horses were present in the graywater-irrigated area of the TX household. A two-way ANOVA was conducted where all of the sampling events were included to evaluate the impact of graywater irrigation on observed *E. coli* numbers. While *E. col*i were detected sometimes in graywater irrigated areas, graywater irrigation was not found to significantly impact observed *E. coli* numbers in soil ($P<0.05$). In 2008, *E.coli* abundance was greater in TX graywater-irrigated soil than in TX freshwater-irrigated soil, with four-fold higher numbers in surface soil (0-15 cm depth) receiving graywater rather than freshwater. Regardless of irrigation water type, *E. coli* numbers were higher in the 30-100 cm depth increment than in the 0-15 cm depth increment in 2008, indicating downward movement of *E. coli* through the soil profile. In 2009, TX soil samples were collected at finer incremental depths, but the abundance of *E. coli* did not increase with depth to the extent it did in

2008. Compared to *E. coli*, numbers of enterococci were highly variable, ranging from below the detection limit to greater than 75,000 g^{-1} soil. Only in CA and in TX 2008 were enterococci numbers greater in graywater-irrigated surface soils compared to freshwater-irrigated surface soil. Results from surface soil samples (0 – 15 cm) did not show a statistically significant impact of graywater irrigation on in enterococci, observed at 6,469±12,145 g^{-1} compared to freshwater-irrigated samples 14,145±30,008 g^{-1} ($P>0.05$). Enterococci appeared to be migrating downwards to at least 30 cm in depth in soil receiving graywater at the TX household, and numbers were greater at the 15-30 and 30-60 cm depth increments in soil receiving graywater compared to freshwater. While enterococci abundance was relatively low in AZ soil receiving graywater, numbers declined even further as distance from the saltbush irrigated with graywater increased. Results from multivariate regression analysis indicate that soil quality parameters had a significant effect on number of indicator organisms (*E. coli* and enterococci), while irrigation type did not significantly affect indicator organism concentration (Table 2-7). OM was the single predictor of *E. coli* abundance, whereas organic matter and percent clay content were best predictors of enterococci abundance. Others have found higher numbers of *E.coli* in organic soils compared to sandy, low organic matter soils and speculated that greater nutrient availability in higher organic matter soils supported the growth, and hence greater abundance, of *E.coli* (Tate, 1978; Ishii et al. 2006). Further research is required to identify soil and environmental factors that best explain *E. coli* and enterococci abundance in soil, however results indicate no consistent increase in these indicator organisms as a result of graywater irrigation. No *Clostridium perfringens* were detected in the AZ, CA, or CO soils, but *Clostridium perfringens* was detected in the freshwater-irrigated surface soil in TX.

Table 2-7. Counts of fecal indicator organisms from soil receiving freshwater or graywater at households with existing graywater systems.

				E. coli	**Enterococci**	***Clostridium perfringens***
Year	**Location**	**Irrigation**	**Depth**	(MPNs g^{-1} soil)		(CFUs g^{-1} soil)
2009	AZ	Freshwater	0-15 cm	<1	75,105	<1
		Graywater	0-15 cm (0-60)	<1	2,091	<1
			0-15 cm (60-90)	<1	1,065	<1
			0-15 cm (90-120)	<1	747	<1
2008	CA	Freshwater	0-15 cm	<1	150	<1
		Graywater	0-15 cm	<1	803	<1
2009	CO	Freshwater	0-15 cm	1	366	ND
			15-30 cm	<1	50	ND
			30-100cm	<1	24	ND
		Graywater	0-15 cm	1	94	ND
			15-30 cm	<1	50	ND
			30-100 cm	<1	<1	ND
2010	CO	Freshwater	0-15 cm	<1	86	ND
			15-30 cm	<1	62	ND
			30-100cm	<1	74	ND
		Graywater	0-15 cm	1	63	ND
			15-30 cm	<1	11	ND
			30-100 cm	<1	23	ND
2011	CO	Freshwater	0-15 cm	<1	30	ND
			15-30 cm	<1	18	ND
			30-100cm	<1	11	ND
		Graywater	0-15 cm	1	43	ND
			15-30 cm	<1	18	ND
			30-100 cm	<1	12	ND
2008	TX	Freshwater	0-15 cm	136	14,000	375
			15-30 cm	43	850	<1
			30-100 cm	216	546	<
		Graywater	0-15 cm	543	31,000	<1
			15-30 cm	160	1,220	<1
			30-100 cm	1,093	2,230	<1
2009	TX	Freshwater	0-15 cm	254	7,768	ND
			15-30 cm	8	6,683	ND
			30-60 cm	36	943	ND
			60-90 cm	75	170	ND
		Graywater	0-15 cm	65	4,764	ND
			15-30 cm	18	8,850	ND
			30-60 cm	<1	1,739	ND
			60-90 cm	<1	65	ND

MPN = most probable number, CFU = colony forming unit, ND = not determined

2.5. Summary of Households

Among the households tested for this study, the TX household appears to be most impacted by graywater, as evidenced by elevated SAR, potentially toxic levels of B, and relatively high numbers of *E. coli* and enterococci. In addition, infiltration rates in the graywater-irrigated soil in TX (95 in h^{-1} 2008 and 16±7.3in h^{-1} 2009) were consistently higher than measured in the freshwater-irrigated area (65 in h^{-1} 2008 and 5.9±3.1 in h^{-1} 2009) at this household. Of note is that the difference in infiltration rate in 2009, when infiltration rates were conducted in three locations in each the graywater and freshwater-irrigated areas, was not statistically significant ($P>0.05$). At the TX household, graywater was applied to the sampled area for more than 30 years through a hose that drained from the washing machine. Soil samples were collected from the area where the homeowner claimed the hose drained to most often over the 30 year period. Graywater was likely applied at rates much higher than required for irrigation in this area. Surfactant concentrations were higher in all graywater-irrigated areas compared to freshwater-irrigated areas at the same household, except at the CA household. However, surfactant concentration in surface samples were not notably different at any one of the households (including TX) compared to other households. Soil analysis from the CA household, where surfactants, SAR, and EC were measured higher in the control area than graywater-irrigated area, was difficult to interpret. This home was located in a highly sloped area with very shallow bedrock. Irrigation water applied likely readily migrated downhill and areas not irrigated with graywater still appeared to be graywater impacted. The AZ and CO households showed lower impact from graywater irrigation compared to the TX household. Of note is that the duration of irrigation was lower (5 years) at these households than the TX household (30 years) and graywater was applied at rates required for irrigation at these households rather than as generated by a washing machine in TX.

2.6. Conclusions

Soil samples were collected from landscape soil irrigated with graywater for more than five years at four households in four different states (AZ, CA, CO, and TX). Analysis of soil samples irrigated with graywater collected from four different households and comparison to control samples irrigated with freshwater provided the ability to determine major concerns for graywater irrigation which may warrant further investigation. Methods for treatment and application of graywater varied from very simple (hose drain from laundry) to more complex and controlled (sand filtration and timed application with submerged drip lines). Results from this study provide an early indication for what can be expected in terms of soil quality changes from application of graywater by individual homeowners. While sodium does appear to accumulate in areas irrigated with graywater, as observed by increased SAR and EC at some sampling locations, this accumulation is not in the range of high concern for plant health and soil quality. Relative infiltration rates were variable among graywater and freshwater-irrigated areas and there is no indication that long term application of graywater results in decreased infiltration. Surfactant concentrations were generally higher in areas irrigated with graywater compared to freshwater-irrigated areas. However, accumulation of surfactants was lower in graywater-irrigated soils than biosolids amended soil. Antimicrobials were only detected in surface soil samples (0-30 cm), indicating that these contaminants do not leach through soil and are not likely to be transported to groundwater. Further investigation is required to evaluate the presence of antimicrobials in graywater-irrigated soil, and to determine the potential effects on microbial communities. No consistent effect of graywater irrigation on numbers of indicator organisms was found. Based on this study, accumulation of salts (as measured by SAR and EC) and surfactants in graywater-irrigated areas do not appear to be a major concern. In addition, graywater irrigation did not result in reduced infiltration rates in soil. Accumulation of B and antimicrobials in graywater-irrigated areas are a potential concern and require further research. Further research including more sample locations and higher numbers of replicates is required to make stronger conclusions regarding the fate of graywater constituents.

Chapter 3

SHORT-TERM EFFECTS OF GRAYWATER IRRIGATION ON ACCUMULATION OF SURFACTANTS AND CHEMICAL CONSTITUENTS IN SOIL[1]

3.1. Introduction

While graywater reuse for household irrigation is widespread, potential effects to soil quality, groundwater quality, and plant health have not been adequately assessed. The application of irrigation water will introduce chemicals to the soil and potentially have short- and long-term effects. This potential depends on application rate, chemical concentrations in the water, biodegradation rate of the chemical, sorption, leaching, and plant uptake. Graywater chemical constituents can potentially migrate to groundwater, surface water, and drinking water sources. In addition, pathogens and viruses present in graywater may persist and pose human health risks. Previous research has not adequately addressed impacts of graywater chemical constituents and pathogens on soil quality, groundwater quality, and plant health. In addition, household graywater has not been adequately characterized. The objective of field experiments was to elucidate information on the fate and occurrence of graywater chemical constituents and their potential impacts on soil quality, groundwater quality, and plant health.

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[1] Will be submitted to the Journal of Ecological Engineering.

3.2. Materials and Methods

3.2.1. Sampling Locations

A total of three households were included in this study. These households installed graywater systems and initiated graywater irrigation during the study period. The households were located in Phoenix, AZ, Cotati, CA, and Fort Collins, CO (Table 3-1). Six sampling events were conducted in AZ, four sampling events were conducted in CA and five sampling events were conducted in CO, respectively (Table 4-1). At these households with newly installed graywater system both soil and graywater samples were collected. At all of the sampling location a baseline sampling event was conducted before initiation of graywater irrigation systems (Table 4-1). At each household, soil samples were collected in areas irrigated with graywater as well as control areas with analogous soil and landscaping that were irrigated with freshwater. Of note is that the source of irrigation water in control areas varied (Tables 3-2).

Table 3-1. Summary of Sampling Events

Location	Sampling Event	Date
Phoenix AZ	Baseline	10/21/2008
	Year 2	6/30/2009
	Year 3	1/12/2010
	Year 3	6/29/2010
	Year 4	3/22/2011
	Year 4	6/27/2011
Cotati, CA	Baseline	9/16/2008
	Year 3	10/27/2010
	Year 4	5/24/2011
	Year 4	10/11/2011
Fort Collins, CO	Baseline	9/28/2009
	Year 3	7/27/2010
	Year 3	9/29/2010
	Year 4	7/07/2011
	Year 4	10/3/2011

3.2.2. Sample Collection

Soil samples were collected with a Zero Contamination sampling tube (0.8 inch diameter) connected to a Backsaver Handle (JMC Soil Samplers, Newton, IA). Sampling tubes were lined with a removable PETG copolyester liner to prevent contamination with surrounding soil as the soil sample was pulled up to the surface. Soil samples were collected as close as possible to the base of a plant that was irrigated with either graywater or freshwater because graywater was typically applied at the plant base. At a minimum, three individual soil samples were collected at each of three depth increments (0-15 cm, 15-30 cm, and 30-100 cm) in households where depth sampling was feasible. Depth sampling was not feasible at the household in CA due to an inpenetrable clay layer. Triplicate samples collected at each location were homogenized in the laboratory manually and treated as one sample. Soil samples were analyzed for pH, electrical conductivity (EC), organic matter (OM), sodium adsorption ratio (SAR), total phosphorus (TP), total nitrogen (TN), ammonia nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), major ions, metals, indicator organisms, surfactants, and antimicrobials. Graywater samples were analyzed for general water quality parameters including pH, EC, TDS, TSS, BOD, COD, TOC, NH_4-N, NO_3-N, PO4-P, SAR, B, indicator organisms including *E. coli*, Enterococci, total coliforms, surfactants including LAS, AES and AE and antimicrobials including TCS and TCC.

3.2.3. Reference Materials

Alkyl ethoxy sulfate (AES) was purchased from Stepan Co (Northfield, IL) in the form of STEOL CS 130, CS 270, and CS 330 respectively. STEOL CS 130, CS 270, and CS330 contain sodium lauryl ether sulfate derived from fatty alcohols that are ethoxylated to an average of 1, 2, and 3 moles respectively. STEPANOL DCFAS-P, which is an alkyl sulfate (AS) with no ethoxylate group, was obtained from Stepan Co (Northfield, IL). NEODOL 25-9®, containing 100% pure polyalcohol ethoxylate (AE), was obtained from Shell Chemical Co, (Houston TX). This product is a mixture of 12 and 13 carbon length alkyl chains with an average of 5 moles ethylene oxide per mole of alcohol ethoxylate. Triclocarban (TCC) and triclosan (TCS) (99% purity) was obtained from Sigma-Aldrich (Milwaukee, WI). Linear Alkylbenzene Sulfonate (LAS), with carbon chain lengths of 10 to 13, were obtained from Proctor and Gamble (Cincinnati, OH). All solvents (methanol, acetone and acetonitrile) were HPLC grade, purchased from Honeywell Burdick and Jackson.

3.2.4. Analytical Methods

Methods for analysis of soil and graywater samples have been outlined in chapter 2, section 2.4 and chapter 4, section 4.3.

3.2.5. Data Analysis

At households with new graywater irrigation systems installed, multiple samples were collected at the same household. To compare the values of means between graywater and freshwater-irrigated areas at these households, a paired t-test was conducted. Population means comparison was conducted by least significant difference (LSD; $P \leq 0.05$). All of the statistical analysis was conducted by XLSTAT software (Addinsoft, New York, NY).

Table 3-2. Summary of graywater irrigation systems at households sampled in this study.

Location	System Description	Irrigation Method	Irrigation Frequency	Source of Irrigation Water in Control Area
Phoenix, AZ	No storage, pumped to roof and gravity feed through gutters	Hose Application	As generated	Municipal and Irrigation Canal Water
Cotati, CA	Subsurface infiltration	Subsurface Infiltration	As generated	Municipal
Fort Collins, CO	Small storage, gravity fed through hose	Hose Application	As generated	Municipal

3.3. Results and Discussion

3.3.1. Graywater Quality

Graywater samples were collected from households with newly installed graywater systems and analyzed for general water quality parameters (Table 3-2) in addition to surfactants and antimicrobials (Table 3-3). AZ graywater included shower water, hand-wash water, laundry water, and kitchen water (no garbage disposal). The CO and CA graywater contained water from showers, bath and hand-wash basins, and laundry. As expected, graywater samples collected from AZ had higher organics and nutrients due to inclusion of kitchen sink and dishwasher water in the graywater (Table 3-3).

Surfactants including linear alkylbenzene sulfonates (LAS), alcohol ethoxy sulfates (AES) and alcohol ethoxylates (AE) were measured in graywater samples. Highly variable concentrations of surfactants in graywater showed that surfactant concentration was affected by types of detergents and personal care products used within each household and can be expected to vary from one site to another (Table 3-4). Trace concentrations of antimicrobials including TCS and TCC were also found in graywater (Table 3-4).

Table 3-3. Graywater quality (n: number of sampling events)

Source	pH	EC (μS cm^{-1})	TDS (mg L^{-1})	Chemical Oxygen Demand (mg L^{-1})	NH_4-N (mg L^{-1})	NO_3-N (mg L^{-1})	PO_4-P (mg L^{-1})	B (mg L^{-1})	SAR
Phoenix, AZ[1]	6.6	1654	930	580	64.9	1.4	18.2	0.04	2.3
Cotati, CA[2]	7.5±0.6	1212±748	571±38	391±13	18.6±1.6	0.9±0.1	8.8±2.0	0.05±0.02	2.8±0.5
Fort Collins, CO[2]	6.7±0.4	945±85	354±92	349±39	15.4±2.0	0.5±0.1	8.7±3.8	0.07±0.03	3.3±0.8

[1] n = 1

[2] n = 3

Table 3-4. Surfactants and Antimicrobials in Graywater Samples. (n: number of sampling events; ND: not detected)

Parameter	AZ (n=1)	CA (n=3)	CO (n=3)
	-------------------- mg L^{-1} --------------------		
LAS (C10-13)	0.7	10.5±2.0	10.0±2.2
AS/AES (EO0-3)	3.9	3.3±0.9	3.5±1.0
AE (C12, EO0-9)	ND	0.8±0.01	0.7±0.2
	-------------------- μg L^{-1} --------------------		
TCS	5.4	6.4±0.7	3.5±1.2
TCC	6.8	8.4±1.0	9.4±4.6

Graywater quality varies from source to source and within a household based on sampling time, location and type of personal care products used at each household (Eriksson et al. 2002). While graywater quality varied at each sampling location, graywater samples collected in this study had total anionic surfactants, TP, TN, pH and chemical oxygen demand (COD) within the same range reported by others (Table 3-5; Wiel-Shafran et al. 2006; Gross et al. 2007; Finely et al. 2009)). The research team observed lower SAR in the graywater samples compared to the SAR values reported by others (Table 3-5; Wiel-Shafran et al. 2006; Gross et al. 2007; Finely et al. 2009). High sodicity of water may cause potential irrigation problems (Ayers and Westcot 1994). According to the Food and Agriculture Organization (FAO) guideline for irrigation water quality there is no degree of restriction associated with reuse of graywater at AZ, CA and CO sampling locations with (Table 3-3; Ayers and Westcot 1994). In addition these graywater sources had B concentration below 0.7 mg L-1, the level which causes toxicity problems in soil (Ayers and Westcot 1994). The only sample collected from the AZ sampling location had TN of 73.8 mg L^{-1}. According to the guidelines for interpretation of water quality irrigation adapted from Ayers and Westcot (1994), this was high above the sever level of restriction on use which is 30 mg L^{-1}. The high level of TN at this sampling location may be caused by mixing of kitchen water with the graywater at this household.

Table 3-5. Typical Household Graywater Quality.

Source	pH	Chemical Oxygen Demand (mg L^{-1})	Total Nitrogen (mg L^{-1})	Total Phosphorus (mg L^{-1})	Sodium Adsorption Ratio	Anionic Surfactants (mg L^{-1})	Reference
Domestic (unspecified)	8.1 ± 0.1	-	19 ± 1.6	31 ± 6	5.9	34 ± 8.2	Wiel-Shafran et al. (2006)
Bath, dish washing and laundry	6.3 - 7.0	702 - 984	25.0 – 45.2	1.72 ± 27	-	4.7 – 15.6	Gross et al (2007)
Shower and laundry	6.7 - 7.6	278 - 435	-	0.24 - 1.2	4.2 - 5.8	-	Finely et al. (2009)
Shower, Hand-wash, bath, laundry	6.3 - 8.1	310 - 580	21.8 - 73.8	4.4 - 16.4	2.3 - 4.1	4.6 - 16.7	Current Study

3.3.2. Soil Quality

3.3.2.1. Accumulation of Salts and B

Water quality data (SAR and EC; Table 3-3) indicated that graywater irrigation water collected from households with newly installed systems had SAR ranged from 2.3-5.9, while EC varied from 900-1700 µS cm^{-1}, and thus would be categorized as none to slight or moderate restrictions for use as irrigation water based (Ayers and Westcot 1994). Among the sampling locations with new graywater systems, highest SAR was measured at the AZ sampling location (Figure 3-1). While SAR was higher than 3 in the soil samples collected from both graywater and freshwater-irrigated soil at this location, no significant difference was observed at this sampling location ($P>0.05$). SAR varied at the CA and CO households and even between different sampling events. No significant difference was observed for SAR in the graywater-irrigated areas compared to the freshwater-irrigated areas at these households ($P>0.05$; Figure 3-1). SAR was measured below 2 in all of the soil samples at these two sampling locations. Similar trends were noted for EC as SAR at these sampling locations (Figure 3-1) and when SAR was higher so was EC. There was no notable increasing trend of SAR or EC in soil with time at any of the three households.

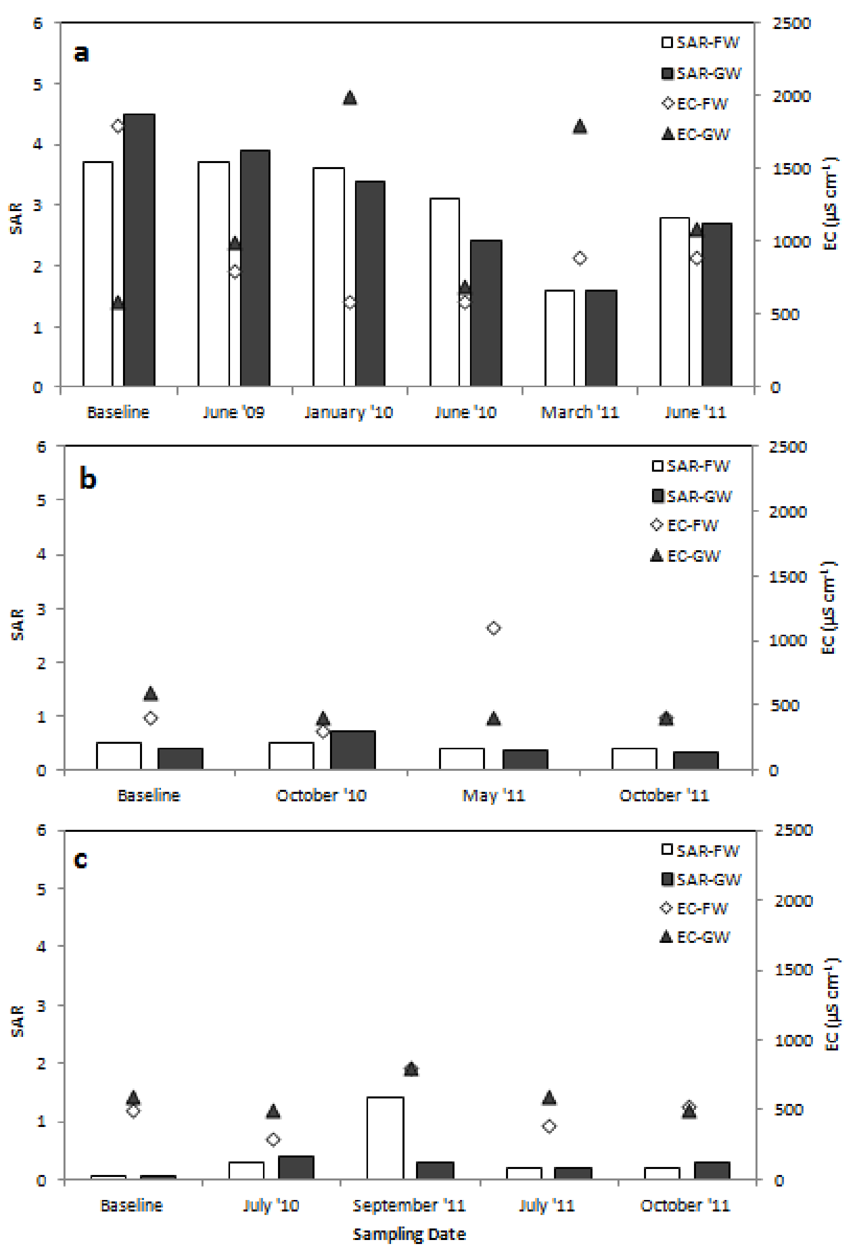

Figure 3-1. SAR and EC in Soil Samples. (a: Arizona, b: California, c: Colorado)

SAR was not found to be statistically different in areas irrigated with graywater compared to freshwater. However, of note is that the longest duration of graywater irrigation at these locations was three years and that may not be enough time for sodium to accumulate in soil. Soil SAR was below 5 at all sampling events, below the threshold for impacts to soil quality and plant health.

Hot water extractable B varied among sampling locations, and even between different sampling events (Figure 3-2). However, no accumulation of B was observed in the areas irrigated with graywater during the course of this field study (Figure 3-2). Results were consistent with data obtained from households which had applied graywater irrigation for more than 5 years (section 2.6.1). B concentrations of 5-8 mg kg^{-1}or higher in soil is considered toxic to many plant types (Nable et al. 1997). Among the sampling events, extractable B concentration in the AZ soil samples in June 2010 was high enough to warrant concern regarding B toxicity to plants. Of note is at this household B level in freshwater-irrigated samples was within the same range of graywater-irrigated samples.

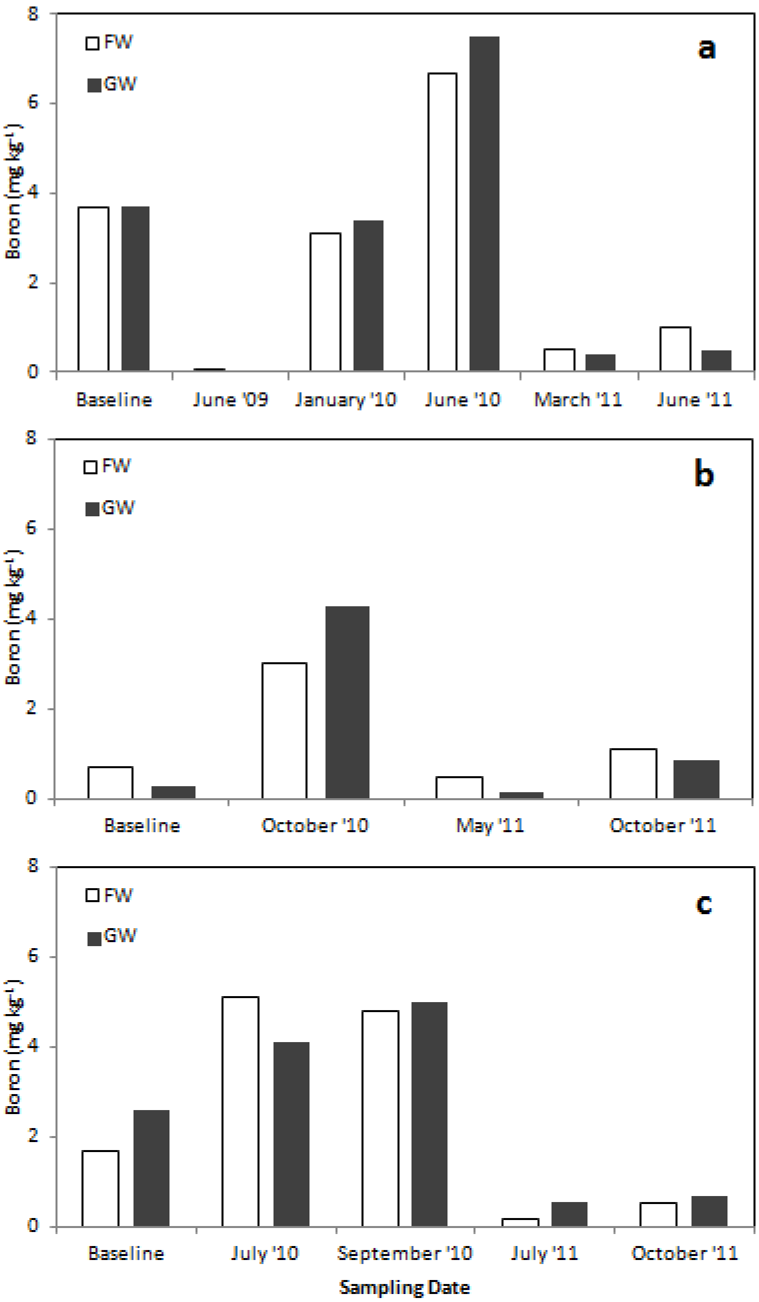

Figure 3-2. B Measured in Surface Soil Samples. (a: Arizona, b: California, c: Colorado)

3.3.2.2. Impact to Nutrients and Organic Content

In AZ and CO, graywater-irrigated surface soil contained 20-50% and 35-53% more OM compared to soil receiving freshwater respectively. OM levels were not notably different at the CA sampling location in the graywater-irrigated area compared to the freshwater-irrigated area.

TN and TP varied among graywater and freshwater irrigated sampling locations and no consistent trend was observed for nutrients.The AZ and CO households with new graywater systems were the only households where soil NO_3-N levels were elevated under graywater irrigation (Figures 3-3). However, these trends also occurred during the baseline sampling events, and thus elevated NO_3-N levels may reflect previous management history or inherent site differences rather than a graywater impact. Within the AZ and CO households with new graywater systems, surface soil NO_3-N content was higher under graywater irrigation (42.6±40.4 and 30.2±15.0 mg kg^{-1}) than under freshwater (13.0±6.2 and 6.8±3.9 mg kg^{-1}) when all samples after graywater irrigation were averaged and this difference at the CO sampling location was significant ($P \leq 0.05$). There was no increasing trend over time of NO_3-N at either of these households.

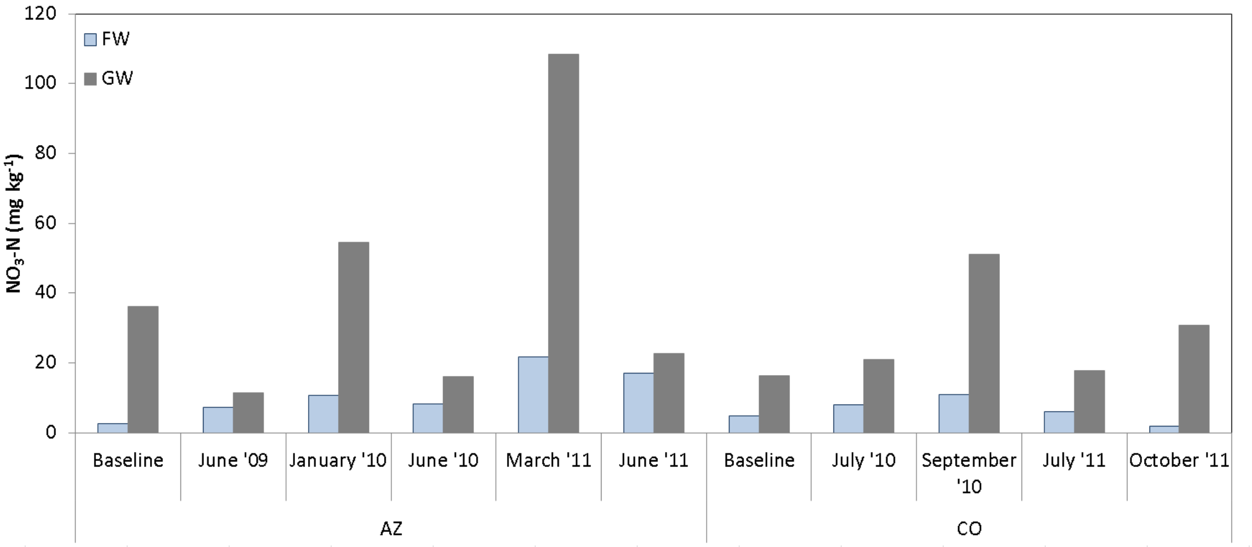

Figure 3-3. Extractable Nitrate Measured in Soil Samples Collected from the AZ and CO Sampling Locations

3.3.2.3.Accumulation of Surfactants and Antimicrobials

Soil samples were analyzed for LAS (C10-13), AES (C12 EO0-3), AE (C12 EO0-9), and fatty acids at each household. Concentrations of LAS (C10-13), AES (C12 EO0-3), and AE (C12 EO0-9) were measured as µmol kg^{-1}, summed and referred to as total surfactants in soil samples. Figure 3-4 summarizes the surfactant concentration in soil samples collected from AZ. At this sampling location, average total surfactants in surface soil samples (0-15 cm) were 453±114 and 122±33 µmol kg^{-1} in graywater-irrigated and freshwater-irrigated areas respectively. Results at this sampling location showed that total surfactants in surface soil samples irrigated with graywater were significantly higher than in soil samples irrigated with freshwater ($P \leq 0.05$). At AZ sampling location, dominant surfactants in depth samples are AES which indicated that they have higher mobility compare to other surfactants. No AE was observed in deep soil (30-100 cm). It can be concluded that AE was most likely adsorbed in the surface soil samples and eventually biodegraded. This result is consistent with other literature as they reported higher hydrophobicity for AE compared to LAS and AES (HERA 2002, 2003, 2009a,b).

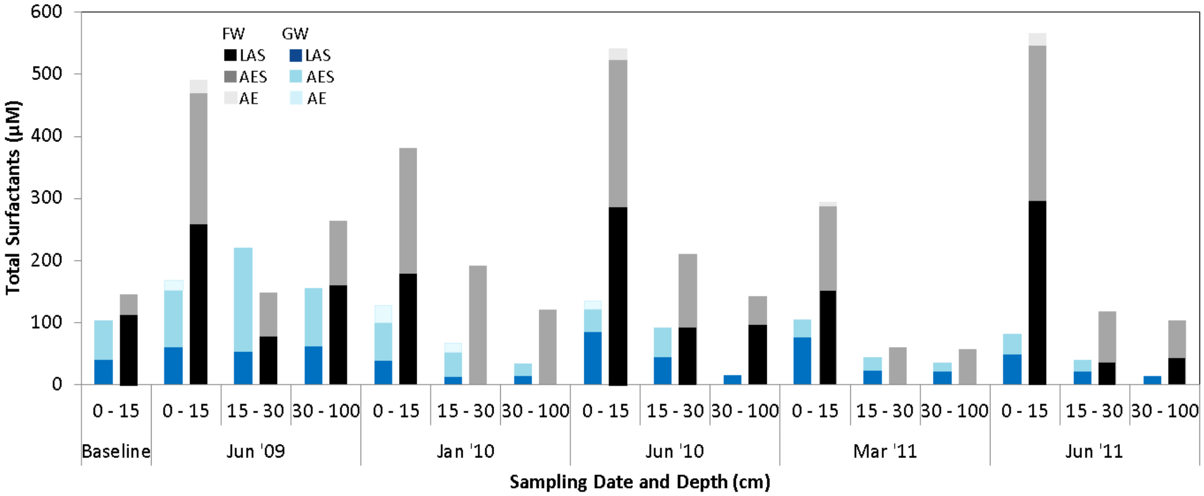

Figure 3-4. Total Surfactants Measured in Soil Samples Collected from AZ Sampling Location

The trend of total surfactants in surface soil samples collected from the AZ household over the course of study was investigated (Figure 3-5). Results showed that after initiation of graywater irrigation, total surfactants in surface soil samples increased from 145 µmol kg^{-1} (baseline sampling event) to an average of 453±114 µmol kg^{-1} over the next five sampling events. Despite the increase of total surfactants in surface soil samples, total surfactants appeared to be higher after the irrigation season (June samples in each year) compared to samples collected over winter months (Figure 3-5). While average total surfactants was 337±63 µmol kg^{-1} in surface soil samples collected in January and March (after the monsoon season and during limited irrigation), average total surfactants were 537±69 µmol kg^{-1} in surface soil samples collected near the end of the dry, intense (June of each year). Results indicated that surfactants substantially increased after graywater irrigation during late spring and summer and then decreased over winter months when irrigation was limitedgraywater irrigation during fall and winter ($P \leq 0.05$; Figure 3-5).

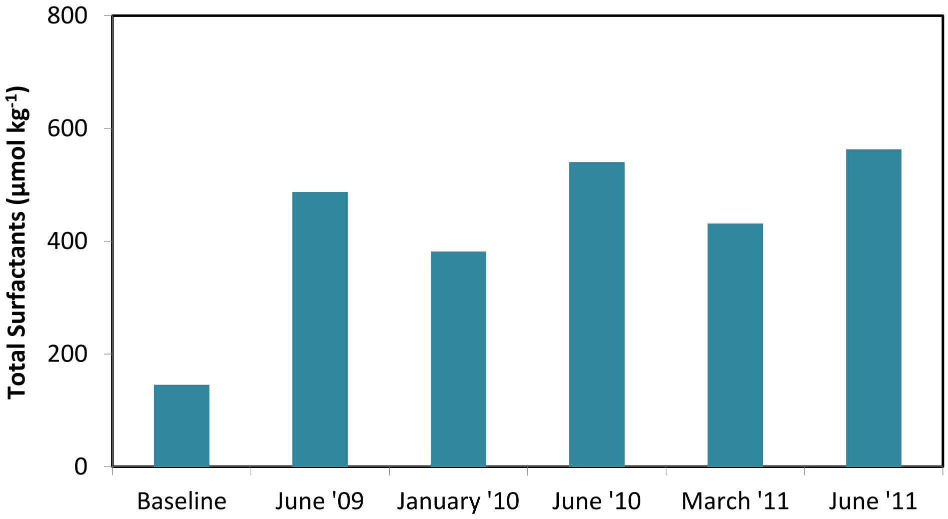

Figure 3-5. Trend of Total Surfactants in Graywater-irrigated Surface Soil Samples Collected from AZ.

In California, depth sampling was not conducted due to limitations described in Section 2.2. Instead, graywater-irrigated soil samples were collected at different distances from the subsurface leach field; approx. 0.6, 2.4 and 4.6 m respectively. At this sampling location, average total surfactants in surface soil samples (0-15 cm) were 280±100 and 98±45 µmol kg^{-1} in graywater-irrigated and freshwater-irrigated soil samples respectively (Figure 3-6). Results indicated that graywater-irrigated soil samples had significantly higher total surfactants than freshwater-irrigated samples ($P \leq 0.05$). In addition, graywater-irrigated soil samples closer to the subsurface leach field (0.6 m) had higher total surfactants (394±77 µmol kg^{-1}) than soil samples collected from further distances (260±23 and 188±36 µmol kg^{-1}; 2.4 and 4.6 m respectively; Figure 3-6). Similar to AZ, surfactants increased after graywater irrigation was initiated, but did not increase notably over time. No AE was observed in the samples collected from 0.6-2.4 and 2.4-4.2 m from the leach field. In addition, results indicated AES had the highest mobility compare to other surfactants as more AES was detected in the soil samples collected from further distance of the leach field (Figure 3-6).

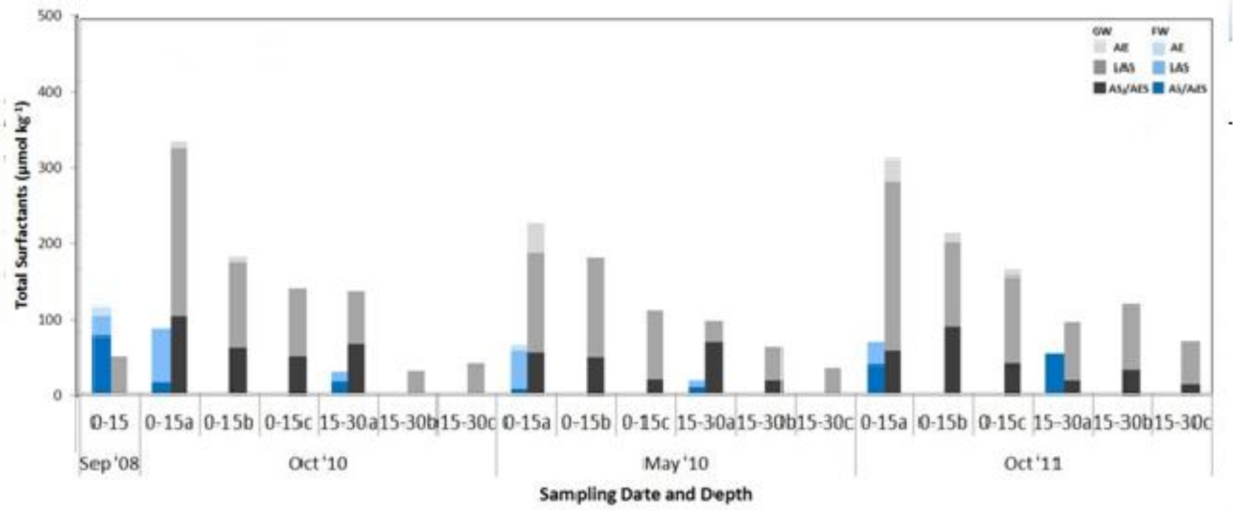

Figure 3-6. Total Surfactants Measured in Soil Samples Collected from CA Sampling Location. (a: 0-0.6, b:0.6-2.4 and c: 2.4-4.2 m distance from the leach field)

In CO, average total surfactants in surface soil samples (0-15 cm) were 556±186 and 129±30 µmol kg^{-1} in graywater-irrigated and freshwater-irrigated areas respectively (Figure 3-7). Results showed that total surfactants in surface soil samples irrigated with graywater were significantly higher than that in soil samples irrigated with freshwater ($P \leq 0.05$), but generally decreased with depth. No AE was observed in deep soil at this sampling location, which was consistent with the result obtained from AZ samples (Figure 3-7). However, notable amount of LAS was detected in depth samples (15-30 and 30-100 cm) at this sampling location compare to AZ samples (Figure 3-7). It should be noted that at this sampling location higher amount of LAS was detected in graywater samples as well which may cause more accumulation of LAS in deep soil compared to other sampling locations (Table 3-5).

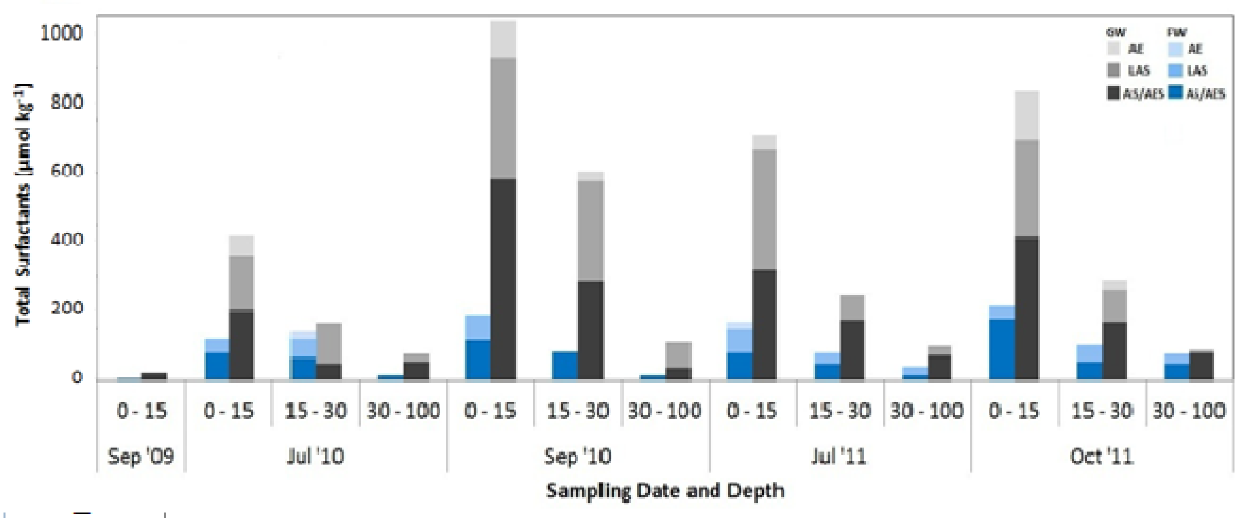

Figure 3-7. Total Surfactants Measured in Soil Samples Collected from CO Sampling Location

The trend of surfactant concentration in surface soil samples over time was evaluated (Figure 3-8). Results showed that after initiation of graywater irrigation, total surfactants in surface soil samples increased from 22 µmol kg^{-1} in baseline sampling event to average of 556±186 µmol kg^{-1} over the next four sampling events. Again, surfactant concentration in soil stabilized over time. Consistent with data collected at the AZ household with a newly installed graywater system, surface soil samples had higher surfactants at the end of the irrigation season (September) than in the middle of the irrigation season (Figure 3-8). While surface soil samples collected in July (2010 and 2011) had 418±154 µmol kg^{-1} total surfactants, soil samples collected at the end of the irrigation season had 695±64 µmol kg^{-1} surfactants ($P \leq 0.05$).

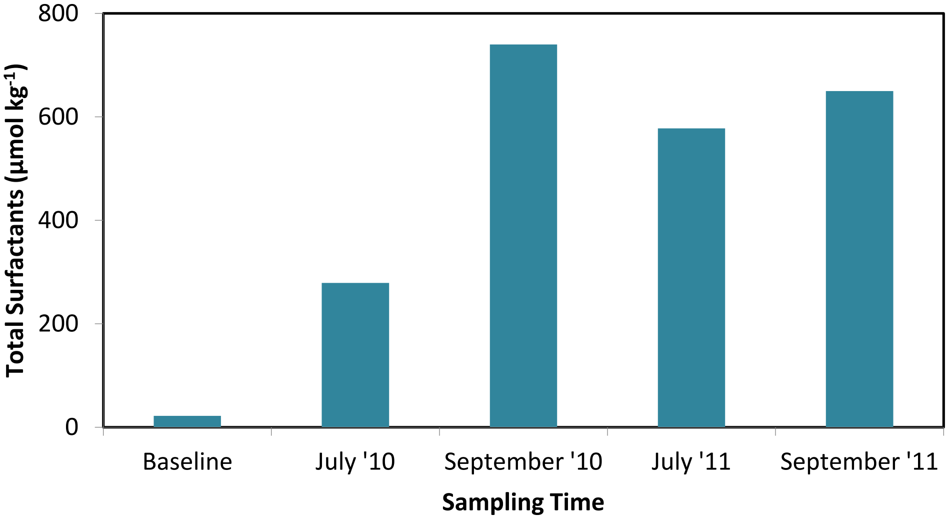

Figure 3-8. Trend of Total Surfactants in Graywater-irrigated Surface Soil Samples Collected from CO.

Surfactant concentrations measured in this study were lower, but comparable to those reported in another study by Travis et al. (2010) where total surfactant was reported to be 0.68±0.39, 0.15±0.06 and 0.53±0.14 mg kg^{-1} in sand, loam and loess irrigated with raw graywater respectively. Results from the households which had graywater irrigation for more than five years also showed an average surfactants concentration of 219±79 µmol kg^{-1} (Chapter 2). Consistent with this result average concentration of total surfactants at the new households was 486±130 µmol kg^{-1}. Results indicated that even after long-term application of graywater for irrigation, surfactant concentration in soil will not increase continuously and will reach constant values.

The relative ratio of surfactants in graywater varied among sampling locations (Figure 3-9), indicating a difference in use of detergents and personal care products at these households. In the graywater samples, LAS, AS/AES and AE were detected in the range of 19.4-71.4%, 22.9-79.0% and 1.6-5.7% respectively (Figure 3-9). Results indicated that surfactant composition varied significantly at the sampling events ($P<0.05$). This variation is may be caused by using different types of products and also depends on the time of sampling. While at the AZ site, AS/AES were the dominant surfactants in the graywater samples (79.0±4.9 %) while at CA and CO households, LAS was the dominant surfactant in the graywater samples (71.4±6.6 and 70±3.7 %). AS/AES were the dominant surfactants in soil samples collected from the AZ and CO locations and LAS was the dominant surfactant in soil samples collected from CA (Figure 3-10). In the soil samples, LAS, AS/AES and AE were detected in the range of 34.3-51.2%, 39.0-57.7% and 3.1-15.8% respectively (Figure 4-9). While surfactant composition varied among different soil samples, this variation was not significantly different ($P>0.5$). At the AZ and CO sampling sites, AS/AES was the dominant surfactants in surface soil samples (57.7±6.2 and 49.9±6.1 %), while at the CA sampling site LAS was the dominant surfactants in surface soil samples (51.2±9.6 %). Overall, the ratio of surfactant species in graywater was not consistent with that observed in surface soil samples (0-15 cm) irrigated with that graywater (Figures 3-9 and 3-10). In addition LAS increased in soil from the ratio observed in graywater in AZ, while the LAS ratio observed in soil decreased from graywater applied for irrigation in CA and CO. These results are difficult to interpret.

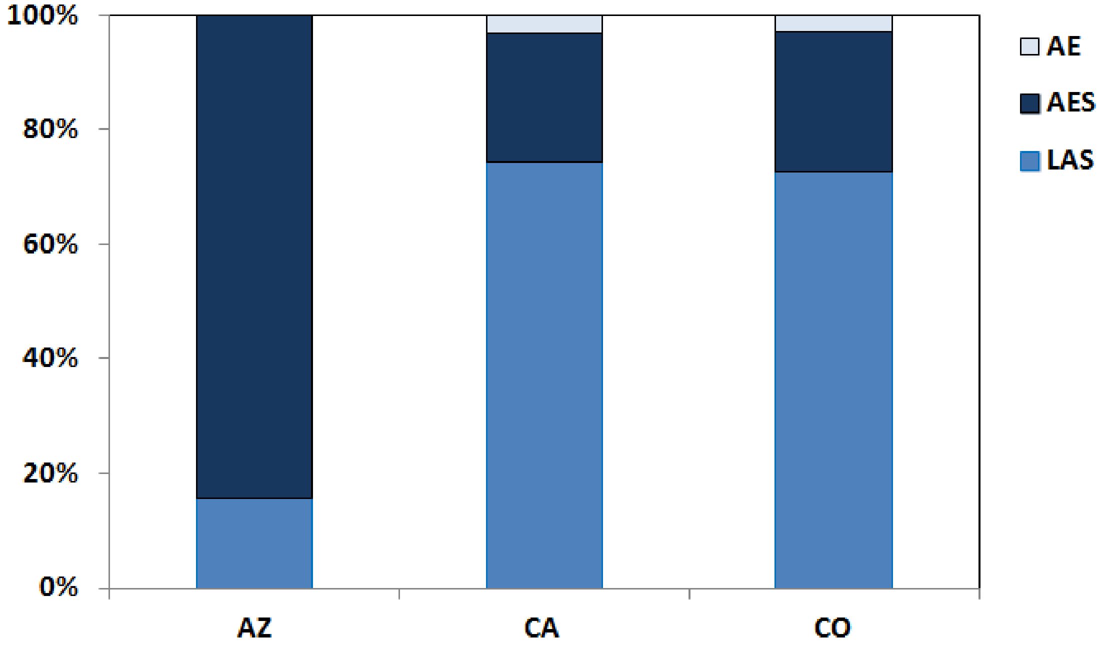

Figure 3-9. Surfactants Composition in Graywater Samples

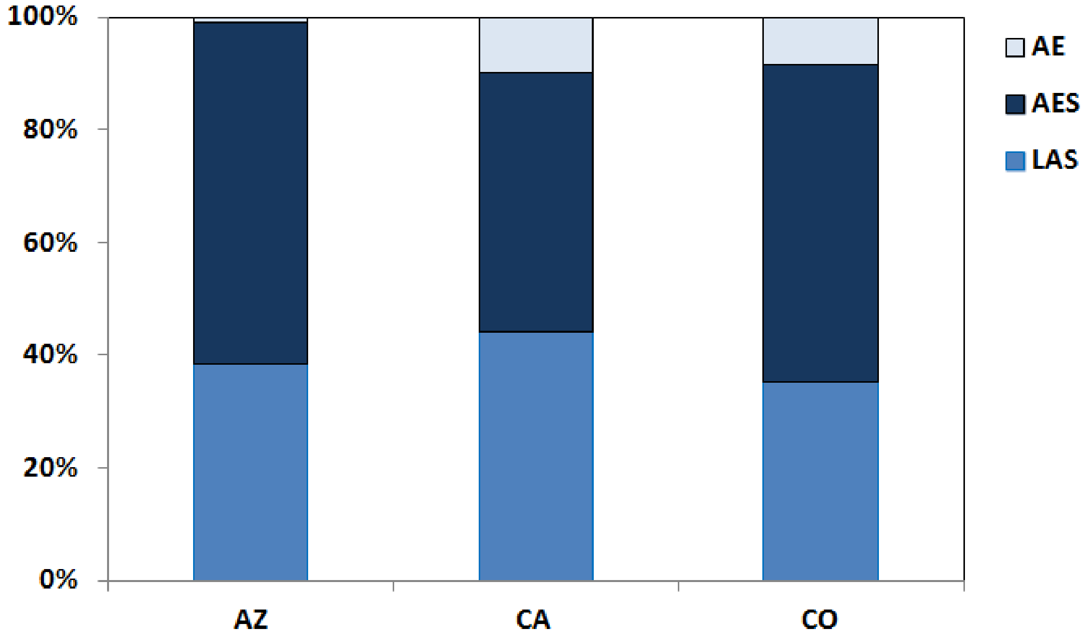

Figure 3-10. Surfactants Composition in Surface Soil (0-15 cm) Samples (calculated as molar mass)

Composition of LAS and AES homologues was evaluated in soil samples at different depths (Figures 3-10 and 3-11). Results showed that among the LAS homologues, LAS C_{12} and among the AES homologues, AS were the dominant homologues.

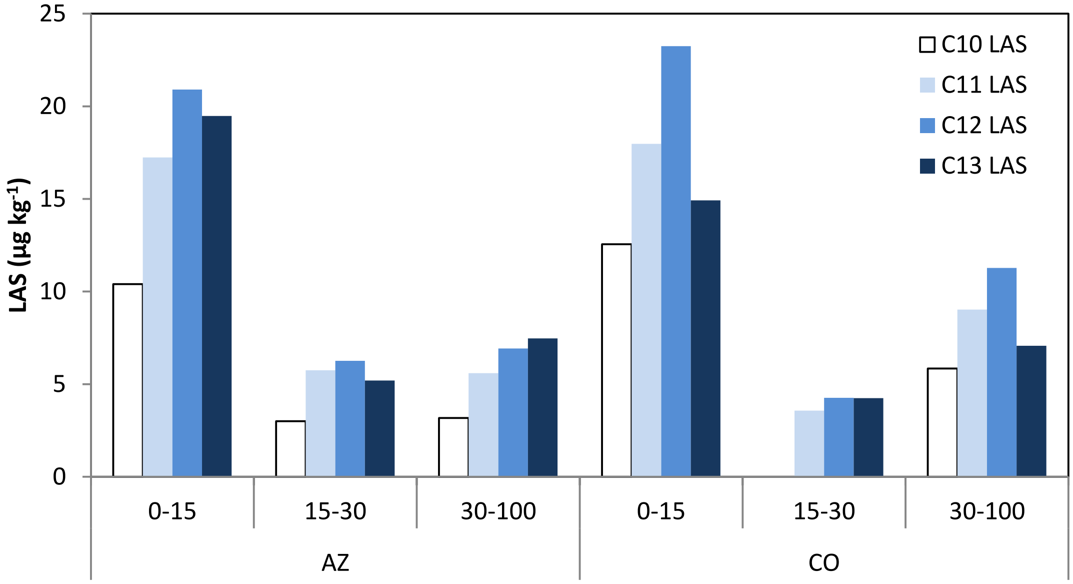

Figure 3-11. LAS Homologues Measured at Different Soil Depths in AZ and CO Sampling Locations

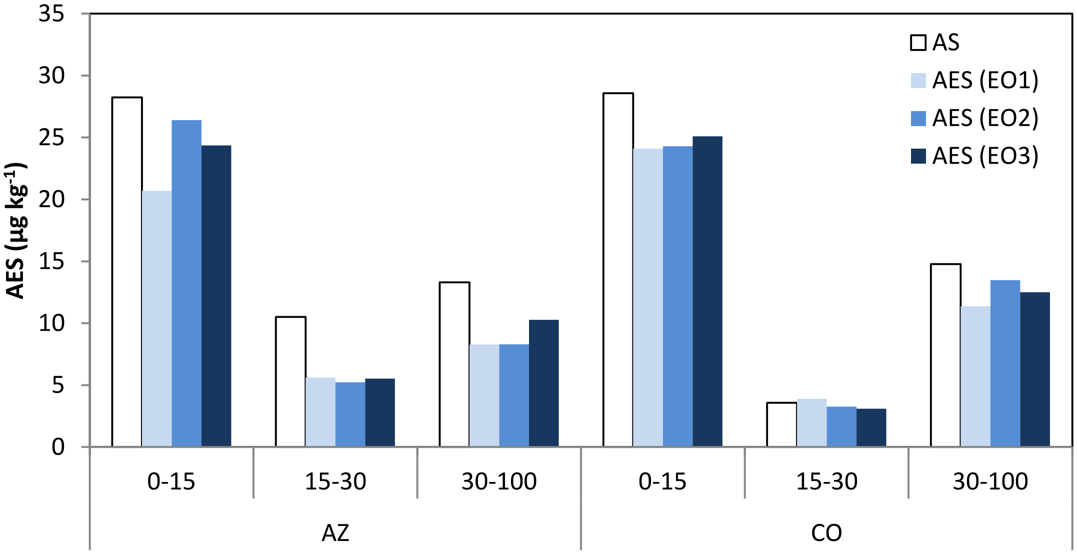

Figure 3-11. AES Homologues Measured at Different Soil Depths in AZ and CO Sampling Locations

Correlation between total surfactant concentration and other major parameters were determined using linear regression analysis (Table 3-6). Results indicated that total surfactants were correlated with TP and OM of the soil especially when the regression analysis was applied at individual sampling locations. While there is limited data on the correlation of phosphorus and surfactants, it has been reported that increase in OM will increase adsorption of surfactants to the soil particles by increasing available sorption sites for hydrophobic components of surfactant molecules (Ou et al. 1996; Yuan and Jafvert 1997; Kuchler and Schnaak 1997). Total surfactants were not significantly correlated with clay content and SAR of the soil (P>0.05). Of note is that total surfactant concentration was correlated with clay content of the soil in the samples collected from existing households.

Table 3-6. Square of Correlation Coefficient, R^2, between Total Surfactants and Other Parameters in Graywater-irrigated Surface Soil Samples

Sampling Location	TP (mg kg^{-1})	OM (%)	Clay (%)	SAR
AZ	0.61	0.06	0.17	0.05
CA	0.5	0.67	0.00	0.07
CO	0.93	0.77	0.03	0.21
All Locations Combined	0.36	0.41	0.02	0.00

The solubility of phosphates is controlled by either sorption-desorption or precipitation-dissolution reactions depending on the environment in the soil or sediments. Phosphorus occurs in nature almost exclusively as phosphate, in all known minerals more specifically as orthophosphate with an ionic form of Pod3 - . The distribution of the different species of orthophosphate is pH-dependent (Holtan et al. 1988). The dissociation of the orthophosphoric acid in aqueous systems as a function of pH, is shown in Figure 3-11. A great part of the phosphorus in soil is sorbed to soil particles or incorporated into soil organic matter. Since phosphorus is also a nutrient it will be found in living organisms.

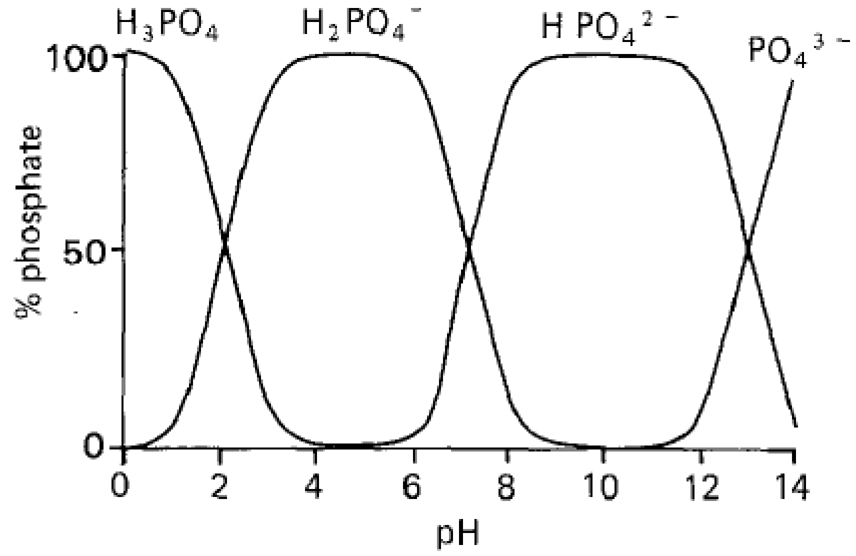

Figure 3-12. Distribution of Phosphate Species with pH (adapted from Holtan et al 1988)

A great part of the phosphorus in soil is sorbed to soil particles or incorporated into soil organic matter. Since phosphorus is also a nutrient it will be found in living organisms. It is very difficult to rank the soil components for their P-sorbing capacity (Holtan et al. 1988). It has been generally agreed upon from kinetic studies that phosphate sorption involves two stages of reaction. The first one proceeds rapidly and may be complete within a few hours while the second may continue slowly for many weeks or months. The time required to establish ‘equilibrium’ depends on the soil constituents and the nature of their surfaces. It is indicated that increase in phosphate sorption with time involved an appreciable shift of phosphate from a more physically sorbed form to a chemisorbed form involving the diffusion of phosphate into structurally porous amorphous material (Berkheiser et al. 1980). The importance of organic matter content on the phosphate sorption is ambiguous. Organic matter can act on phosphate sorption in two ways, either by sorbing phosphate or by blocking sorption sites on inorganic particles (Halten et al. 1988).

Soil samples were analyzed for antimicrobials TCS and TCC (Table 3-7). Antimicrobials were below the detection limit (0.4 µg kg^{-1} and 0.2 µg kg^{-1} for TCS and TCC respectively) in all soil samples collected from freshwater irrigated areas. Except for two sampling events in AZ, antimicrobials were only detected in surface soil samples (0-15 cm). Among the six sampling events conducted in AZ, antimicrobials were only detected in depth samples (15-30 cm) for two events (1.0 µg kg^{-1} TCS in June 2009 and 3.5 µg kg^{-1} TCC in January 2010).

Table 3-7. Antimicrobials Measured in Surface Soil Samples (0-15 cm) Irrigated with Graywater.

Sampling Location	Sampling Date	TCS (µg kg^{-1})	TCC (µg kg^{-1})
AZ	Jun '09	3.8	6.3
	Jan '10	5.6	7.1
	Jun '10	6.3	10.2
	Mar '11	2.1	2.8
	Jun '11	8.2	9.3
CA	Oct '10	6.7	4.2
	May '11	6.1	5.2
	Oct '11	9.8	4.5
CO	Jul '10	0	0
	Sep '10	4.2	8.7
	Jul '11	3.3	9.7
	Sep '11	6.5	8.9

Even though antimicrobials were primarily detected in surface soil samples (0-15 cm) collected, the concentration of TCS and TCC were notable in those areas where detected. A concern associated with high concentrations of antimicrobials in soil would be decreased microbial activity. One indicator of decreased microbial activity may be higher surfactant concentration in those areas where TCS and TCC were detected, which was not noted here. Further investigation is warranted to determine the effect of graywater irrigation on antimicrobial concentration in soil and the impact this may have to soil microbiology and the potential formation of antibiotic resistant genes.

Statistical analysis was conducted individually at each sampling location on major parameters to evaluate the effect of graywater irrigation on accumulation of chemical constituents in surface soil samples (Table 3-8). Results indicate that graywater irrigation significantly increased surfactant concentration in soil samples received graywater at all sampling location ($P \leq 0.05$). No significant effect of graywater irrigation was observed on SAR and pH of the soil ($P > 0.05$). While no significant effect was observed on TN, and TP concentration at AZ and CA sampling locations ($P > 0.05$), graywater-irrigated soil samples had significantly higher TN and TP at CO sampling location ($P \leq 0.05$). An ANOVA test with no replication was conducted at all sampling locations (combined) to investigate the effect of graywater application on surface soil quality (Table 3-9). Result indicated that graywater irrigation significantly increased surfactant concentration, TIN, OM and significantly decreased pH of the surface soil samples ($P \leq 0.05$; Table 4-8).

Table 3-8. Results of Statistical Analysis for Different Parameters in Surface Soil Samples Collected After Initiation of Graywater Irrigation (GW: graywater-irrigated, FW: freshwater-irrigated; a, a: not significantly different ($P>0.05$); a, b: significantly different ($P\leq0.05$))

	Surfactants (µM kg^{-1})		SAR (-)		B (mg kg^{-1})		pH	
Location	**GW**	**FW**	**GW**	**FW**	**GW**	**FW**	**GW**	**FW**
AZ	453±114^{a}	122±33^{b}	2.8±0.9^{a}	3.0±0.8^{a}	2.4±3.2^{a}	2.3±2.7^{a}	7.3±0.2^{a}	7.6±0.1^{b}
CA	526±215^{a}	119±26^{b}	0.5±0.2^{a}	0.5±0.1^{a}	1.8±2.2^{a}	1.5±1.3^{b}	6.2±0.4^{a}	6.4±1.0^{a}
CO	457±142^{a}	114±33^{b}	0.3±0.1^{a}	0.5±0.6^{a}	2.6±2.3^{a}	2.6±2.7^{a}	7.3±0.2^{a}	7.6±0.2^{a}

	TIN (mg kg^{-1})		TN (%)		TP (mg kg^{-1})		OM (%)	
	GW	**FW**	**GW**	**GW**	**GW**	**FW**	**GW**	**FW**
AZ	47.4±40.6^{a}	16.3±5.4^{a}	0.23±0.07^{a}	0.20±0.05^{a}	77.3±59.0^{a}	69.5±55.1^{a}	4.2±0.6^{a}	2.8±0.8^{b}
CA	6.3±3.8^{b}	18.3±5.8^{a}	0.17±0.02^{a}	0.20±0.03^{a}	131.1±41.5^{a}	136.4±45.6^{a}	3.4±1.1^{a}	4.2±0.8^{a}
CO	34.0±15.7^{a}	9.8±4.2^{a}	0.28±0.03^{a}	0.21±0.03^{b}	131.0±53.4^{a}	39.8±40.2^{b}	5.7±1.3^{a}	3.2±0.8^{b}

Table 3-9. Factor ANOVA Evaluating Significance Difference of Treatments (Irrigation Water: graywater vs. freshwater) and Sampling Events Where 12 Sampling Events Were Evaluated (GW: graywater-irrigated, FW: freshwater-irrigated; *: statistically significant at 95% confidence intervals, $P \leq 0.05$)

Parameter	GW	FW
Surfactants (μM kg^{-1})	472±140*	119±29
SAR	1.4±1.4	1.5±1.4
Boron (mg kg^{-1})	2.2±2.3	2.3±2.5
TIN (mg kg^{-1})	32.7±30.0*	14.6±5.9
TN (%)	0.23±0.06	0.20±0.04
TP (mg kg^{-1})	108.6±55.9	76.3±58.4
OM (%)	4.5±1.3*	3.3±0.9
pH	7.0±0.6*	7.3±0.7

3.4. Conclusion

Result of this study indicated that SAR was not found to be statistically different in areas irrigated with graywater compared to freshwater. However, of note is that the longest duration of graywater irrigation at these locations was three years and that may not be enough time for sodium to accumulate in soil. Soil SAR was below 5 at all sampling events, below the threshold for impacts to soil quality and plant health. B varied among sampling locations, and even between different sampling events. However, no accumulation of B was observed in the areas irrigated with graywater during the course of this field study. Graywater-irrigated samples collected from AZ and CO sampling locations with newly installed graywater systems had significantly higher nitrate than freshwater-irrigated soil samples. OM was variable among sampling locations for both the existing households and new installations and there was no indication that graywater irrigation impacted OM.

Results showed that surfactants accumulated in soil samples especially in the surface soil due to irrigation with graywater. However, based on results from the households with new installations, graywater irrigation resulted in increased surfactant from the baseline sampling event and then did not increase with duration of irrigation. Surfactant concentrations remained fairly constant over time with some decreases after rainy seasons. Results also indicated that while AE was only detected in surface soil samples, considerable amount of LAS and AES was observed in depth soil samples. Based on the results obtained from this study, surfactants with lower carbon chain length had higher leaching potential than surfactants with higher carbon chain lengths.

Even though antimicrobials were only detected in surface soil samples (0-15 cm) collected, the concentration of TCS and TCC were notable in those areas where detected. Further investigation is warranted to determine the effect of graywater irrigation on antimicrobial concentration in soil and the impact this may have to soil microbiology and the potential formation of antibiotic resistant genes.

Chapter 4

LEACHING POTENTIAL OF CHEMICAL CONSTITUENTS IN SOIL-PLANT SYSTEMS IRRIGATED WITH GRAYWATER[1, 2]

4.1. Introduction

Recycling of graywater is becoming increasingly considered a component of integrated urban water management, particularly in regions with limited water resources. Graywater, when defined as wastewater from laundry, showers, baths, and wash basins, has been estimated to account for nearly 50% of total wastewater generated within a household (Mayer et al. 1999). Because graywater can be reused for nonpotable uses with little or no treatment, graywater reuse has the potential to achieve substantial water conservation, offering particular benefit in arid and semi-arid regions. One approach that is gaining popularity is application of graywater for landscape irrigation. Graywater reuse for landscape irrigation requires simple treatment systems compared to reuse of graywater for toilet flushing, where human contact with water is likely and disinfection is typically recommended. However, even with the fast growing momentum of graywater reuse for landscape irrigation, more studies are required to ensure safe practices. Effects of graywater application to soil quality and leaching of graywater constituents into groundwater are both concerns which must be addressed (Roesner et al. 2006; Misra and Sivonxay 2009; Pinto et al. 2010; Negahban-Azar et al. 2012).

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[1] Masoud Negahban Azar did the experiment set up, run the experiments, performed sampling, conducted physical, chemical, surfactants and antimicrobial analysis, conducted the statistical analysis and performed the whole manuscript. Sybil Sharvelle supervised all phases of the project and edited the text. Yaling Qian performed the plant analysis. Alicia Shogbon contributed to the experiment setup, sampling and analysis.

[2] Submitted to Journal of Science of the Total Environment.

Graywater contains varying levels of organic matter, nutrients, salts, suspended solids and pathogens. Due to variation in water uses and products within households, graywater quality varies from source to source and also within a source based on both when and where the sample was collected (Eriksson et al. 2002). When graywater is reused for irrigation, constituents of concern to soil quality and potential leaching to groundwater resources include organic content, nutrients (nitrogen (N) and phosphorus (P)), metals, salts, boron (B), and personal care product ingredients).

In recent years, several studies have been conducted to evaluate effects of graywater irrigation on soil quality (Jeppesen 1996; Blevins and Lukaszewski 1998; Misra and Sivongxay 2009; Mahler 2009; Pinto et al. 2009; Travis et al. 2010). Jeppesen (1996) reported that graywater may contain elevated levels of sodium. Laundry graywater in particular contains elevated sodium, and if it is not well balanced with Ca and Mg (high sodium adsorption ratio [SAR]), hydraulic conductivity of soil may be reduced (Misra and Sivongxay 2009). In another study, Negahban-Azar (2012) reported that while SAR was generally higher in areas irrigated by graywater compared to freshwater, SAR was not high enough to be of concern for soil quality. B is another concern because it is toxic to plants when presents in irrigation water (Mahler 2009; Blevins and Lukaszewski 1998). Negahban-Azar et al. (2012) reported a potential increase in B concentration of the soil after long-term application of graywater for irrigation and at some sites was measured above toxicity threshold concentration for plant health. Pinto et al. (2009) reported no significant differences in total N and P in soils irrigated with graywater compared to soil irrigated with potable water. While studies have shown that surfactants are present in higher concentrations in graywater irrigated areas compared to areas irrigated by potable water, the degree of accumulation of surfactants reported in soil is variable (Wiel-Shafran 2006; Travis et al. 2010; Negahban-Azar et al. 2012). These studies were conducted under field conditions and conclusions on fate and transport of chemical constituents differed, likely a result of the difficulty in interpreting data collected in the field.

Another important concern associated with graywater application for landscape irrigation is leaching of chemical constituents through soil and ultimately into groundwater. Studies to date have not addressed this concern. However, reclaimed water reuse for irrigation has been largely applied for decades and many studies have been conducted on the leachability of various constituents after application of recycled water for irrigation. While some of these studies suggested that reclaimed wastewater may enhance soil quality by adding nutrients and OM to the soil, researchers recommended risk assessment to be conducted prior to irrigation with reclaimed water to ensure safe application of treated wastewater (Candela et al. 2007; Kalavrouziotis et al. 2008; Gharbi Tarchouna et al. 2010; Xu et al. 2010; Chavez et al. 2011). Leaching of nitrates is one of the greatest threats to groundwater quality arising from reclaimed water irrigation due to its high solubility (Bond et al. 1998; Hermon et al. 2006). Candela et al. (2007) reported higher values of nitrate in groundwater samples under reclaimed wastewater irrigated areas compared to areas irrigated with potable water. Graywater contains less N than residential wastewater not only because it does not contain urine, which is the main source of nitrogen, but also because usually kitchen water is excluded from graywater. Leaching of nitrogen from graywater application may be of lower concern than reclaimed wastewater because nitrogen concentrations are lower in graywater (0.6-21 mg L^{-1} total nitrogen (TN) if kitchen water is excluded; Eriksson et al. 2002) than reclaimed water, except when the wastewater treatment process includes denitrification. Leaching of B and salts are other concerns associated with reuse of reclaimed wastewater for irrigation. Stewart et al. (1990) observed long-term changes in soil pH as a result of displacing cations or excessive leaching of them into deeper horizons caused by reclaimed wastewater irrigation. In another study, no B retention was observed in soil-turf filters irrigated with reclaimed wastewater, which indicates high leaching potential for B (Anderson et al. 1981). While little is known on fate and leaching of personal care products, few studies raised the concern over leaching of xenobiotic compounds and emerging contaminants into groundwater due to reclaimed wastewater application (Pedersen et al. 2005; Chefetz et al. 2008; Xu et al. 2009).

Application of graywater for irrigation has become more widespread only in recent years and scientific data is not available on the fate and leaching potential of graywater chemical constituents after graywater irrigation. While studies to date have addressed the impact of graywater irrigation on soil quality, leachability of graywater constituents has not been studied. Furthermore, many questions are still unanswered concerning long-term changes in soil physical and chemical properties and accumulation of salts, surfactants and other chemical constituents. Given the large number of variables in the field, a more controlled environment is required to draw comprehensive conclusions about fate and leachability of graywater constituents after application. The objective of this study was to examine the fate of graywater chemical constituents in soil-plant systems under controlled conditions. For this research, a set of columns with sandy loam soil and different plant types was performed in a greenhouse. Four different plant species were studied and leachate water quality was analyzed. In addition, soil samples were collected at termination of the experiment, after seventeen months of continuous irrigation with synthetic graywater, and analyzed to determine fate of graywater constituents, accumulation of chemicals and changes in soil physical and chemical properties.

4.2. Materials and Methods

4.3.1. Reference Materials and Reagents

Alkyl ethoxy sulfate (AES) was purchased from Stepan Co (Northfield, IL) in the form of STEOL CS 130, CS 270, and CS 330 containing sodium lauryl ether sulfate derived from fatty alcohols ethoxylated to an average of 1, 2, and 3 moles respectively. STEPANOL DCFAS-P, which is an alkyl sulfate (AS) with no ethoxylate group, was obtained from Stepan Co (Northfield, IL). NEODOL 25-9®, containing 100% pure polyalcohol ethoxylate (AE), was obtained from Shell Chemical Co, (Houston TX). This product is a mixture of 12 and 13 carbon length alkyl chains with an average of 5 moles ethylene oxide per mole of alcohol ethoxylate. Linear Alkylbenzene Sulfonate (LAS), with carbon chain length of 12 was obtained from Stepan Co (Northfield, IL) in the form of Bio-soft D-40. All solvents (methanol, acetone and acetonitrile) were HPLC grade, purchased from Honeywell Burdick and Jackson (Morristown, NJ).

4.3.2. Experimental Setup

Columns were setup containing plants and synthetic graywater and potable water were applied for irrigation. A sandy loam soil (Pioneer Sand Company, Fort Collins, CO) was used for experiments with a composition of 65% sand, 17% silt and 18% clay (Table 4-1). Prior to the start of the experiments, the sandy loam soil was analyzed to determine the soil physico-chemical properties and background concentrations of constituents of concern (Table 4-1).

Table 4-1. Initial Soil Quality

Parameter		Parameter	
Sand (%)	65	CEC (meq/100g)	12.17
Silt (%)	17	NH_4-N (ppm)	1.9
Clay (%)	18	NO_3-N (ppm)	13.1
pH	7.5	TP (ppm)	14
EC (µS cm^{-1})	1300	B (mg kg^{-1})	1.1
OM (%)	1.5	SAR	4
LAS (µg kg^{-1})	3.5	Total C (%)	0.589
AE (µg kg^{-1})	ND	Total N (%)	0.034
AES (µg kg^{-1})	ND		

Polyvinyl chloride (PVC) pipes (61 cm, schedule 40; Kelly Supply Co., Fort Collins, CO) were used to construct columns (Figure 4-1). Columns were exposed to sunlight for six weeks prior to initiation of experiments to minimize degradation of PVC and subsequent release of toxins into plant columns during the experiment. The setup of the 6 in. diameter plant columns involved a two-chamber system in which the top 22 inches encased the soil and plant biomass and the bottom 2 inches served as a drainage layer (Figure 4-1). During the course of experiments, two different species of grasses and shrubs were studied. The turfgrasses used were bermudagrass (a warm season grass) and tall fescue (a cool season grass) and were selected because they are common in arid regions. The shrubs used were Meyer Lemon and Emerald Gaiety Euonymus. During a field study conducted on impacts of graywater to soil quality and plant health (Negahban-Azar et al 2012), Euonymus was observed to be tolerant to graywater irrigation, while Lemon trees indicated sensitivity to graywater irrigation. Plants were selected to study a variety of species observed to be sensitive and insensitive to graywater irrigation. Soil in columns was compacted to a bulk density of 1.5 g/cm^3, the compaction ratio typically used in lab-scale column studies (Abu-Zreig et al 2003). A total of 38 plant columns were constructed to hold the soil and plants (and in some cases only soil) and set up in the Colorado State University (CSU) Greenhouse (Plant Growth Facilities) for experiments. The greenhouse temperature was controlled between 20-25°C through all seasons during the course of the experiments. Eight columns were planted with each plant species and six columns were left unplanted as controls (containing only soil). Of the eight columns used for each plant, four were irrigated with graywater and the other four were irrigated with potable water. Of the six columns set up without plants, three were irrigated with potable water and three were irrigated with synthetic graywater. To ensure initial plant growth and health, fertilizer was added to the containing plants at the initiation of the experiments. Osmocote (Osmocote Technologies, Marysville, OH) indoor and outdoor smart-release fertilizer with 19% of nitrogen was used and 0.47 g was added to potable water irrigated columns. The fertilizer amounts were adjusted for graywater-irrigated plants based on the nitrogen content of graywater used for irrigation and the amount of graywater expected to be added. Adjusted amounts of fertilizer were 0.32, 0.30, 0.31, and 0.35 g for tall fescue, bermudagrass, Euonymus, and Lemon respectively. The duration of the experiment was seventeen months from February 2010 to June 2011.

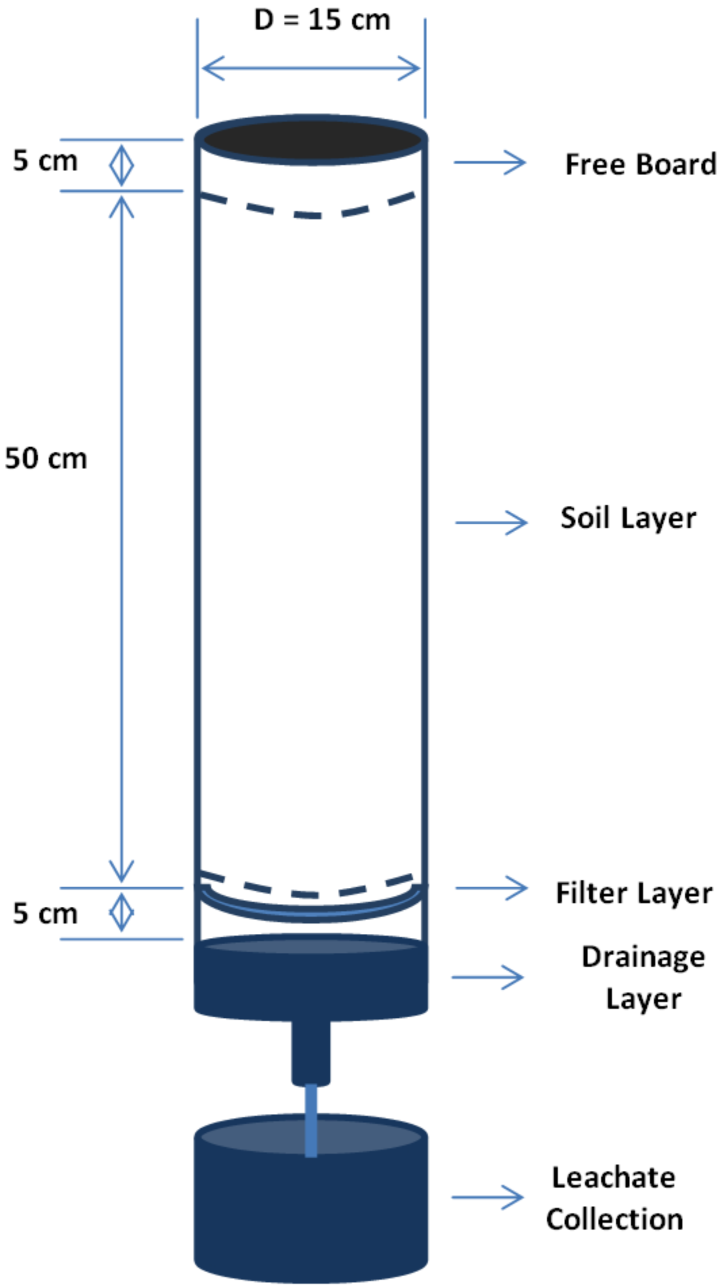

Figure 4-1. Schematic of column setup.

4.3.3. Synthetic Graywater

The synthetic graywater (Table 4-2) was formulated to contain constituents typically found in graywater sources (Gerba et al 1995, Christova-Boal et al 1996, Finley et al 1998, Friedler 2004, Ramon et al 2004, Gross et al 2005, Shafran et al 2006, Roesner et al 2006, Pidou et al 2008). Synthetic graywater was composed in D.I. water on daily basis and applied immediately for irrigation in order to avoid any changes in composition. Yeast extract was included in the synthetic graywater solution to simulate organics in graywater of which the source is not surfactants. Yeast extract is commonly applied to simulate organic matter in graywater (Fenner and Komvuschara 2005; Panikkar et al 2010). The quality of irrigation water is reported in Table 4-3.

Table 4-2. Synthetic Graywater Recipe

Reagent	Concentration (mg L^{-1})
Ammonium Chloride Crystalline	8.5
Sodium Nitrate	15.8
Sodium Borate	4.4
Potassium Phosphate monobasic	3.5
Magnesium Sulfate Anhydrous Powder	57.3
Potassium Chloride Crystalline	11.4
Calcium Chloride	47.1
Sodium Chloride	25.6
Calcium Sulfate	143.3
Sodium Sulfate	40.5
LAS (C_{12})	21.0
AES ($EO_{0\text{-}3}$)	4.5
AE	0.9
Yeast Extract	248.3

Table 4-3. Synthetic graywater and potable water analysis (NM-not measured)

Parameter	**Synthetic graywater**	**Potable water**
pH	7.4	7.1
EC (μS cm^{-1})	1050	120
TDS (mg L^{-1})	609.5	73
COD (mg L^{-1})	378	NM
TIN (mg L^{-1})	4.8	0.18
TP (mg L^{-1})	0.8	NM
B (mg L^{-1})	0.5	≤0.1
Alkalinity (mg/L as $CaCO_3$)	158	30
SAR	0.8	NM

4.3.4. Irrigation Procedure

Irrigation water was applied manually and the amount was determined based on the evapotranspiration (ET) rate and plant (or grass) type. To estimate the ET rate in the greenhouse and subsequently determine irrigation scheduling, a black bellani plate atmometer was used (Robertson and Holmes 1957). The ET for the plant was calculated as described by Equation 4-1 with the appropriate plant coefficient (coef.).

Equation 4-1.

$$(ET_{plant} = coef. \times ET_{plate})$$

Crop coefficients were applied from Allen et al. (1998) and were 0.85, 0.95, 0.90 and 0.65 for bermudagrass, tall fescue, Lemon and Euonymus respectively. The irrigation amount for each plant and grass was different and was set as 120% of the plant ET to ensure leachate generation. The plants were potted in October 2010 and irrigated with potable water for the first five months of the study. This irrigation was continued for the potable water-irrigated plants through the end of experiments in June 2011. However, synthetic graywater irrigation for the graywater-irrigated plants was started in February 2010 and continued for seventeen months until June 2011.

4.3.5. Leachate Collection

Throughout the experiment, the total volume of generated leachate was recorded. In addition, there were five sampling events for leachate water quality in April, June and August of 2010 and January and May of 2011. Leachate samples were collected from three out of four plant columns with the same irrigation method as replicates. At least 250 mL of leached water was collected immediately after irrigation in glass sampling bottles and transferred to the laboratory for immediate analysis. To assess leachate quality, several water quality parameters were measured including dissolved organic carbon (DOC), TN, nitrate (NO_3-N), ammonium (NH_4-N), total phosphorous (TP), B, SAR, electrical conductivity (EC), LAS, AES, AS, AE, total dissolved solids (TDS). Leachate samples were filtered (1.5 µm) before DOC, TP, TN, nitrate, ammonium, and surfactant analyses. NH_4-N and NO_3-N were summed to report total inorganic nitrogen (TIN).

4.3.6. Soil sample collection and Plant Biomass Production

At the termination of experiments, soil samples were collected from depths of 0, 25 and 45 cm from the top of each column. Three holes were drilled into the walls of the columns exactly at the center of the horizontal axis and approximately 100 g of soil was collected with an aluminum soil corer and placed in glass sampling bottles. Soil samples were analyzed for physico-chemical parameters including pH, EC, organic matter (OM), SAR, B, NO_3-N, NH_4-N, TP and surfactants (LAS, AES and AE).

Irrigation was terminated in June 2011 and plants were removed and analyzed for above ground biomass. Aboveground biomass of the plants was removed at the end of experiments. Plants’ biomass was oven-dried (at 70ºC for 48 h) and weighed to measure the biomass for each plant types. Grasses were trimmed when needed during the experiments and biomass was measured.

4.3.7. Analytical Methods

Leachate Samples: DOC and TN present in leachate samples were measured using TOC-VCSH organic carbon analyzer (Shimadzu, Columbia, MD). TP, TDS, TSS and VSS were measured according to Standard Methods for the Examination of Water and Wastewater (APHA and AWWA 2005). Nitrate and ammonium were measured using O.I Analytical Flow Solution 3000 ion analyzer (O.I. Analytical, College Station, TX). Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, Cl^{-}, B, and sulfate were determined using Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES; TJA Solutions IRIS Advantage High Resolution Radial View) following acid digestion of the leachate samples with nitric acid and hydrochloric acid. The conductivity and pH were measured using a conductivity meter (Thermo Orion 145A+; South Burlington, VT). Surfactant extraction was carried out using solid phase extraction (SPE) with Waters OASIS hydrophilic-Lipophilic Balance [HLB] cartridges based on the manufacturer’s recommended method. To begin, the Oasis HLB extraction cartridges were placed on a vacuum manifold and the vacuum was set to 5 in. Hg. The cartridges were first conditioned by adding and drawing through the cartridge 1 mL of methanol. To equilibrate the system, 1 mL of HPLC-grade water was added and drawn through the cartridges. A sample volume of 1 mL was then added and drawn through the cartridges. To wash out the cartridges, 1 mL of 5% methanol in water (v/v) was added and drawn. Then the collection vessels were replaced with clean ones and collected liquids were discarded. Finally, 1 mL of methanol was added and drawn through with the cartridges, and then extracts were collected in vials and analyzed using liquid chromatography mass spectrometry (LC-MS) An Agilent 1200 (Agilent Technologies, Santa Clara CA) high-throughput HPLC system coupled with an Agilent 6220 Accurate Mass Time of Flight mass spectrometer (Agilent Technologies, Santa Clara CA) was used for determination of LAS, AS/AES, and AE using the same analytical method as described by Negahban-Azar et al. (2012). Recovery tests were performed at the beginning of the experiments and the recovery percentage for the SPE was always above 92%.

Soil Samples: Soil texture was determined on each sample using the hydrometer method described by Gee and Bauder (1986). Soil pH was determined by the saturated paste method (Method 21a of USDA Handbook No. 60, 1954) and soil EC was determined by the saturated paste method of Rhoades (1996). OM was determined by the modified Walkley-Black method described by Nelson and Sommers (1996). Concentrations of Mg^{2+}, Ca^{2+} , K^+ and Na^+ ions were analyzed on an inductively coupled plasma-atomic emission (ICP) spectrophotometer (Thermo Jarrell Ash Corp., Franklin, MA). The saturated paste extraction method was used for the ions as described by Sumner and Miller (1996). B concentration was determined by the hot water extraction method, followed by ICP analysis (Keren 1996). NO_3-N and NH_4-N were measured using in-line UV/Persulfate digestion and oxidation with flow injection analysis and ammonia-selective electrode method respectively as outlined in as outlined in Greenberg et al. (1992). NO_3-N and NH_4-N were summed and are reported throughout as TIN. TP was determined using the AB-DTPA method developed by Soltanpour and Schwab (1977). Percent OM was determined by the modified Walkley-Black method described by Nelson and Sommers (1996).

Extraction and analysis of surfactants from soil samples was conducted based on methods described by Negahban-Azar et al. (2012).

4.3.8. Data Analysis

Because evapotranspiration (ET) occurred in columns, it was necessary to evaluate constituents in terms of mass loading and output rather than concentration only. The amount of TIN and surfactant leached from the columns was determined as percentage by mass of the applied amount. The volume of synthetic graywater applied for irrigation to each column was used to calculate the mass loading rate of each constituent. In addition, the leached rate of each constituent was measured as mg day^{-1} based on concentration in the leachate and the volume of the leachate collected. Leached percentage by mass was calculated (Equation 2):

Equation 2.

$$Leached\ Percentage = \frac{\left(leached\ rate, \frac{mg}{d}\right)}{\left(loading\ rate, \frac{mg}{d}\right)} \times 100$$

To better evaluate the fate of TIN and surfactants after application of synthetic graywater for irrigation, a mass balance was conducted for these constituents. For simplicity, plant uptake and fertilizer addition were neglected in the mass balance. For each TIN and surfactants the concentration in the soil samples, loading rate and leaching rate were considered. Simplifying assumptions were applied to perform the mass balance. The average porosity of sandy loam used in the columns was assumed as 0.43. It was assumed that TIN and surfactant concentration varied linearly between sampling depths. The loaded values for TIN and surfactants were determined based on volume of synthetic graywater applied for irrigation to each column during the course of experiment and their concentration in synthetic graywater. Due to variation of leaching rate of TIN and surfactants, the averaged leached amount from the first three sampling events were used for the first 10 months of the experiments. Then, the averages of the leached percentage from the last two sampling events were used for the last 7 months of the experiments.

Statistical packages SPSS 5.0 for WINDOWS (SPSS Inc., Chicago, IL) and Microsoft Excel for Windows (Microsoft Corporation, Redmond, WA) were used for statistical calculations. Population means comparison was conducted by least significant difference (LSD; $P \leq 0.05$). Testing for trends was conducted using regression method ($P \leq 0.05$). Data comparison and testing for trend were conducted based on the methods described by EPA guidance for data quality assessment (EPA 2000).

4.4. Results and Discussion

4.4.1. Leachate Volume

The volume of generated leachate was monitored through the experiment duration. In February 2010, irrigation with graywater was initiated for columns designated for synthetic graywater irrigation. Starting from March 2010, lower leachate generated from the graywater-irrigated columns (19.8±11.2 % of applied water collected as leachate; Figure 4-2) than that generated from the potable water-irrigated columns containing plants (42.8±18.9 % of applied water collected as leachate; Figure 4-2). The trend of lower leachate volume for the graywater-irrigated columns compared to the potable water-irrigated columns was observed for planted columns (Figure 4-2). This might be due to higher water uptake rate for graywater-irrigated plants than potable water-irrigated plants. Data from above ground biomass analysis revealed that graywater-irrigated plants had higher above ground biomass than potable water-irrigated plants ($P \leq 0.05$; Figure 4-3). It is thought that higher nutrient loading in graywater-irrigated columns, in particular nitrogen (further discussion to follow), resulted in higher growth rate for graywater-irrigated plants. Of note is that there was no significant difference in leachate generated in unplanted columns irrigated with potable water (25.4±0.6%) compared to graywater (26.1±1.4%; P>0.05), which supports the hypothesis that plant growth contributed to higher water uptake in planted graywater irrigated columns. The presence of roots in planted columns likely resulted in preferential flow paths increasing liquid flow through the columns. Of note is less leachate was collected in late spring and summer (April through July in 2010 and March through May in 2011) due to longer daylight hours and higher plant growth, which caused higher uptake of water.

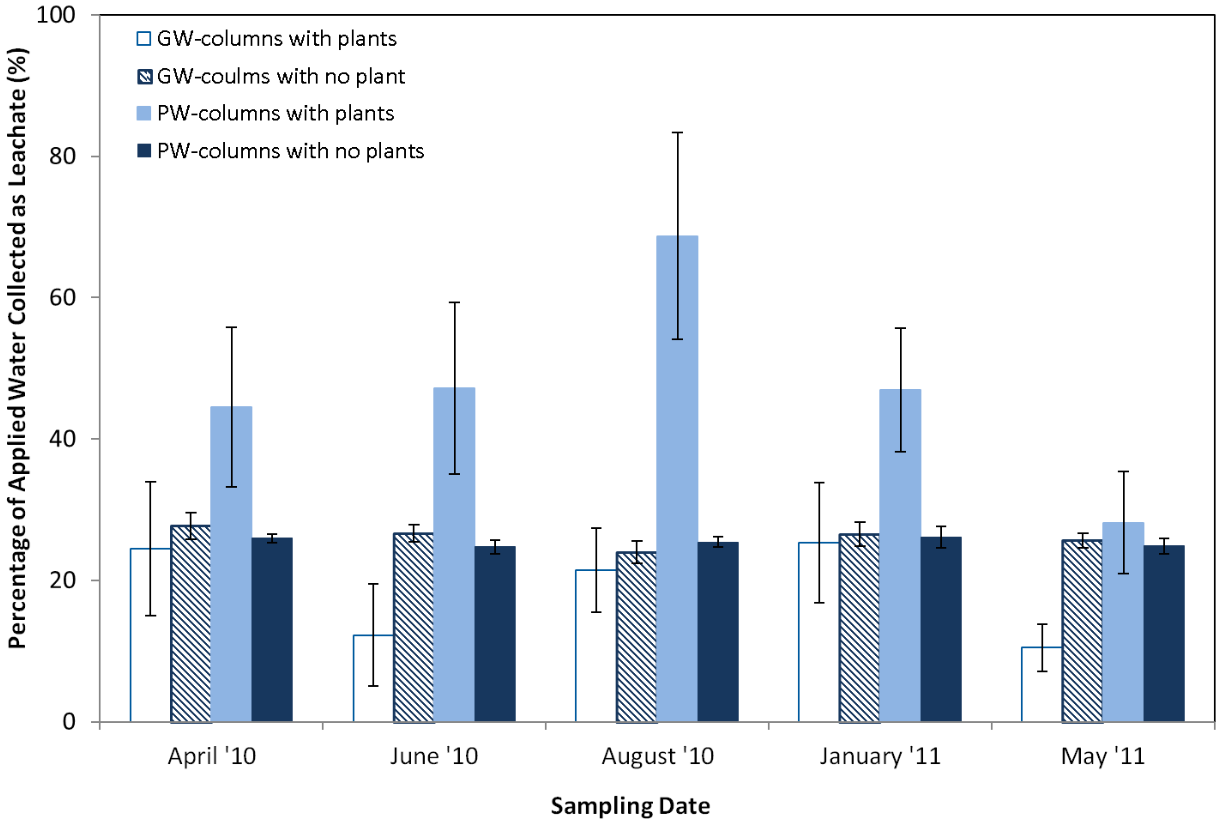

Figure 4-2. Leachate Volume Collected as Percentage of Applied Water. (GW: graywater-irrigated, PW: potable water-irrigated)

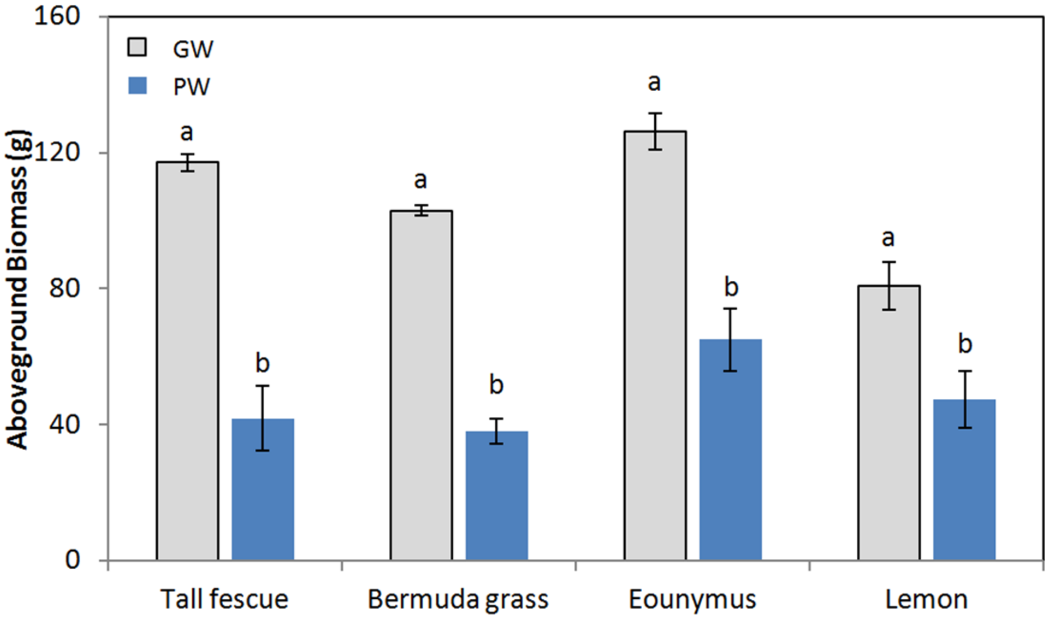

Figure 4-3. Above Ground Total Biomass Measured at the End of Study. (a, b: significantly different ($P \leq 0.05$); a, a: not significantly different ($P > 0.05$); GW: graywater-irrigated, PW: potable water-irrigated)

4.4.2. Salts and B

Leachate quality analysis revealed that TDS leached from the graywater-irrigated columns (60.0±36.5 mg day^{-1}) was significantly higher than TDS leached from potable water-irrigated columns (27.4±16.1 mg day^{-1}; $P \leq 0.05$) for all sampling events (Table 4-4). The higher TDS leached from the graywater-irrigated columns than that in potable water-irrigated columns was expected due to the higher input of salts from the graywater (Table 4-3). In addition SAR in the leachate samples collected from planted and unplanted columns was higher in graywater irrigated columns (2.6±1.4) than potable water irrigated leachate (1.0±1.0). Result from measurement of TDS in the leachate samples was consistent with EC and SAR observed in soil samples collected at the termination of the study (Table 4-4). EC values measured in soil samples showed a significant difference between graywater-irrigated and potable water-irrigated soil samples for all samples collected (different depths and plants; Table 4-4). Of note is that higher

EC values were detected in depth soil samples (45 cm) than surface samples in all columns except for Lemon and unplanted columns irrigated with graywater (Table 4-5). SAR was also generally high in the depth soil samples (45 cm). This in conjunction with higher TDS leached from graywater-irrigated columns may raise concern over the potential leaching of salts to groundwater sources when graywater is applied for irrigation. When comparing TDS leached from columns with plants irrigated with graywater (Figure3-4), TDS leached from columns planted with tall fescue was higher than TDS input to the system for January and May (2011) samples. This is likely a result of accumulation of salts in soil over the duration of the study (Table 4-5) and subsequent leaching of the salts. The greater TDS leached in the latter part of the experiment from columns planted with Tall Fescue also suggests that salts accumulated in the soil during the first 300 days of the experiment. Also of note is that fertilizer was applied to columns at the beginning of the experiment, which contributed additional salts, and leachate collected from potable water-irrigated columns containing plants had TDS values (29.8±14.4 mg day^{-1}) higher than the applied rate in potable water (5.9±2.8 mg day^{-1}).

Sodium accumulation has been a problem for reclaimed water irrigation and is also a concern for graywater irrigation (Qian and Mecham 2005; Candela et al. 2007; Gharbi-Tarchouna et al. 2010). Graywater SAR is expected to range from 4.2-5.9 (Wiel-Shafran et al. 2006; Finely et al. 2009). Soil analysis for SAR revealed that graywater-irrigated soil samples had significantly higher SAR values (1.0±0.3) than potable water-irrigated soil samples (0.5±0.0; $P \leq 0.05$; Table 4-4). The structure of some soils can be adversely affected by sodium when SAR levels are more than 5 (Mace and Amrhein 2001). SAR in the soil columns generally decreased from the initial value of 4 and was far below 5 in all soil samples regardless of type of irrigation.

Table 4-4. Comparison of constituents leached from columns with graywater-irrigation (GW) and potable water-irrigation (PW) where mass leached was averaged over columns for the four plants studied (n=3; a, b: significantly different $P \leq 0.05$; a, a: not significantly different $p > 0.05$)

Parameter	**April'10**		**June'10**		**August'10**		**January'11**		**May'11**	
(mg day^{-1})	**GW**	**PW**	**GW**	**PW**	**GW**	**PW**	**GW**	**PW**	**GW**	**PW**
TDS (mg day^{-1})	83.0^{a}	34.0^{b}	45.6^{a}	39.7^{a}	58.0^{a}	35.1^{b}	71.3^{a}	30.6^{b}	60.1^{a}	12.5^{b}
B (mg day^{-1})	0.02^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.03^{a}	0.01^{b}	0.05^{a}	0.01^{a}	0.05^{a}	0.004^{b}
DOC (mg day^{-1})	0.4^{a}	0.4^{a}	1.8^{b}	4.5^{a}	0.5^{a}	0.8^{a}	0.5^{a}	0.5^{a}	0.5^{a}	0.4^{a}
TIN (mg day^{-1})	0.07^{a}	0.06^{b}	0.03^{a}	0.04^{a}	0.02^{a}	0.01^{b}	0.05^{a}	0.00^{b}	0.01^{a}	0.00^{b}

Of note is that SAR in the synthetic graywater was 0.8, lower than typically observed in graywater (4.2-5.9; Wiel-Shafran et al. 2006; Finely et al. 2009). Lower SAR values in the synthetic graywater compared to real graywater may have resulted in less change in soil SAR than would be observed if real graywater were applied for irrigation. However, SAR observed in field samples irrigated with graywater for more than five years was 1.3±1.1 (Negahban-Azar et al. 2012). Other researchers have reported that short-term graywater application led to an increase in soil SAR but not to a detrimental level (City of Los Angeles 1992; Travis et al. 2010; Negahban-Azar et al. 2012).

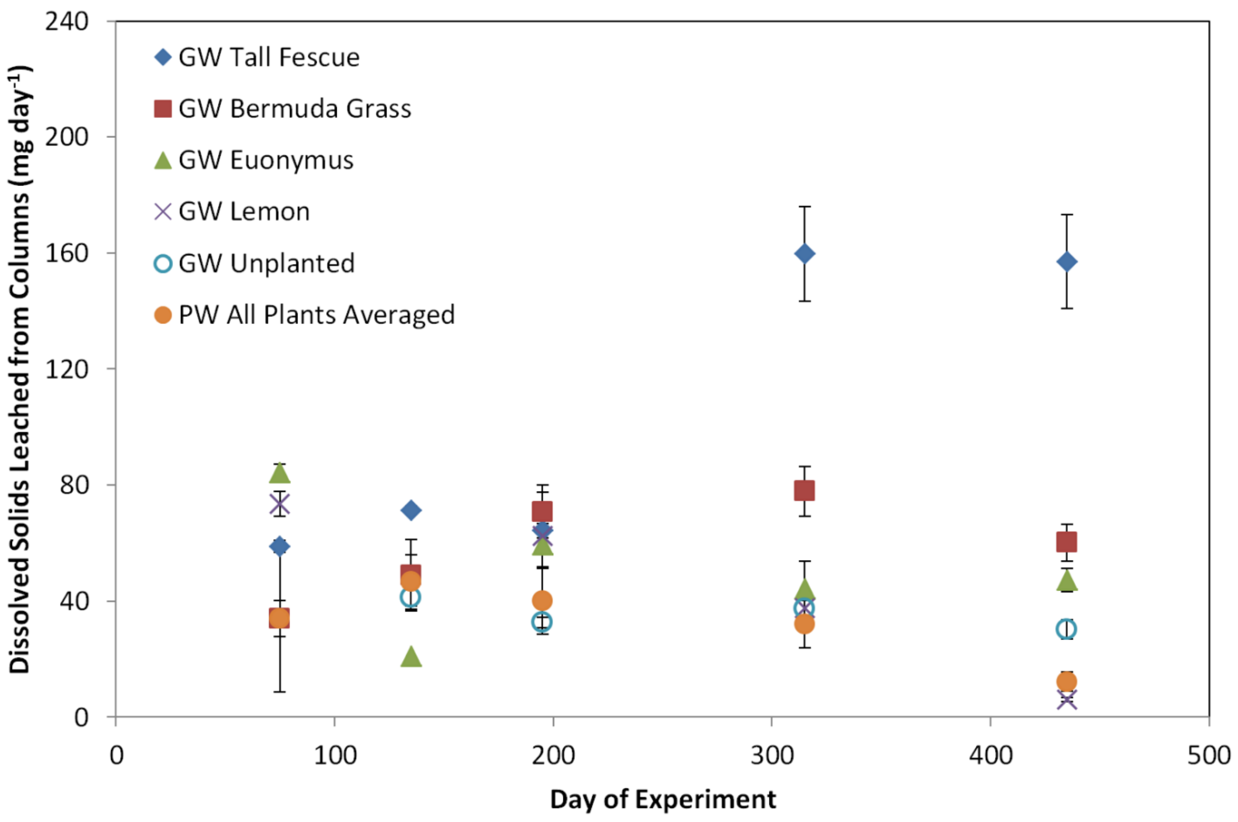

Figure 4-4. Total Dissolved Solids Leached from Columns. (average input TDS was 138±8, 98±7, 135±9, 101±7, and 107±7 mg day^{-1} for tall fescue, Bermuda grass, euonymus, lemon and unplanted columns respectively; no leachate sample was collected from lemon columns in June sampling event; GW: graywater-irrigated; PW: potable water-irrigated)

B is of concern because it is toxic to plants (Mahler 2009; Blevins and Lukaszewski 1998). In the current study, B leached from columns was consistently significantly higher in the columns irrigated with graywater compared to columns irrigated with potable water (Table 4-4). In addition, B leached from columns was considerably higher in January and May samples compared to earlier samples (Figure 4-5). However, B was also consistently present in the leachate collected from columns irrigated with potable water (Table 4-4), likely a result of its presence in the soil used for this study (1.1 mg kg^{-1}; Table 4-1). B leached from columns increased over the duration of the study (Figure 4-5).

Table 4-5. Salts and B Measured in Soil samples Collected from Different Depths (Numbers are averaged, n = 3, GW: graywater-irrigated; PW: potable water-irrigated; a, b: significantly different P≤0.05; a, a: not significantly different P>0.05)

	Depth	B (mg kg^{-1})		EC (µS cm^{-1})		SAR	
Plant Type	**(cm)**	**GW**	**PW**	**GW**	**PW**	**GW**	**PW**
Tall Fescue	0	2.2^{a}	0.5^{b}	348^{a}	367^{a}	1.4^{a}	0.6^{b}
	25	1.8^{a}	0.4^{b}	420^{a}	250^{b}	2.0^{a}	1.0^{b}
	45	3.6^{a}	0.4^{b}	912^{a}	250^{b}	1.8^{a}	0.6^{b}
Bermuda Grass	0	2.9^{a}	0.6^{b}	690^{a}	280^{b}	1.0^{a}	0.5^{b}
	25	1.9^{a}	0.7^{b}	420^{a}	165^{b}	1.4^{a}	0.6^{b}
	45	3.2^{a}	0.6^{b}	984^{a}	216^{b}	1.7^{a}	0.6^{b}
Euonymus	0	2.3^{a}	0.3^{b}	740^{a}	210^{b}	0.7^{a}	0.5^{a}
	25	2.1^{a}	0.4^{b}	588^{a}	248^{b}	1.4^{a}	0.7^{b}
	45	2.6^{a}	0.4^{b}	888^{a}	200^{b}	2.2^{a}	0.6^{b}
Lemon	0	1.9^{a}	0.7^{b}	572^{a}	213^{b}	0.9^{a}	0.5^{b}
	25	1.1^{a}	0.4^{b}	412^{a}	152^{b}	1.3^{a}	0.7^{b}
	45	1.4^{a}	0.5^{b}	510^{a}	268^{b}	1.6^{a}	1.0^{b}
No Plant	0	1.3^{a}	0.4^{b}	378^{a}	156^{b}	1.0^{a}	0.5^{b}
	25	0.9^{a}	1.0^{a}	264^{a}	144^{b}	1.0^{a}	0.7^{b}
	45	0.9^{a}	0.6^{b}	240^{a}	168^{b}	1.0^{a}	0.7^{b}

Consistent with these results, no B retention was observed in soil-turf filters irrigated with reclaimed wastewater, which indicates high leaching potential (Anderson et al. 1981). A trend analysis was conducted on average B concentration in the leachate collected from graywater-irrigated plants over the period of the study. Result showed a significant increasing trend in B leached from the graywater-irrigated columns over the duration of the study ($R^2=0.84$; $P \leq 0.05$). Hot water-extractable B is a good indicator of plant available B. In this research, graywater-irrigated soil samples had significantly higher B concentration than potable water-irrigated in nearly all soil samples ($p \leq 0.05$; Table 4-5), which showed accumulation of B in graywater-irrigated soil. However, results revealed that after 15 months of continuous graywater irrigation, B levels in soil samples were still below the deteriorative levels of 5 mg kg^{-1} (Nable et al. 1997) with a maximum of 2.9 mg kg^{-1} (Table 4-5). Results from a study on long-term effect of graywater application for landscape irrigation also indicate that irrigating with graywater can increase B concentration in soil (Negahban-Azar et al. 2012).

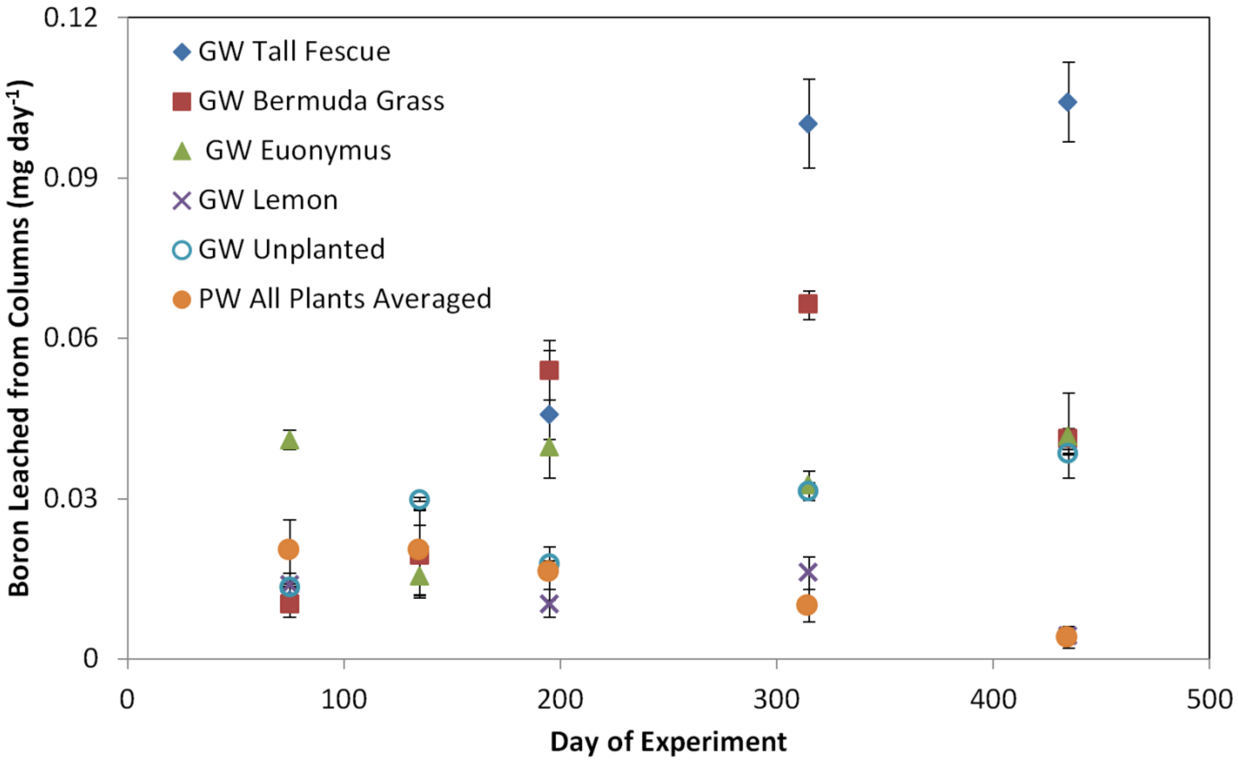

Figure 4-5. B Mass Leached From Graywater-irrigated Columns. (average input B was 0.11, 0.08, 0.11, 0.083 and 0.088 mg day^{-1} for tall fescue, Bermuda grass, euonymus, lemon and unplanted columns respectively; no leachate sample was collected from lemon columns in June sampling event; GW: graywater-irrigated; PW: potable water-irrigated)

4.4.3. Nutrients

The post-application fate of N in synthetic graywater irrigation involves several storage pools, transfers and transformations. Synthetic graywater contains inorganic forms of nitrogen species NH_4-N and NO_3-N. Soil initially had some organic-N, which can be microbially mineralized to NH_4. Ammonium can engage in soil cation exchange reactions, volatilize to the atmosphere as NH_3 under appropriate conditions of soil pH, immobilize to organic nitrogen or oxidize to NO_3-N through the action of nitrifying bacteria in aerobic soil zones. Nitrate can be reduced to N_2 (and to a much lesser extent N_2O) by denitrifying bacteria in anaerobic microzones, with the gaseous end products again lost to the atmosphere. Inorganic-N (NO_3 and NH_4 +) is available for assimilation into microbial or plant biomass. All organic and inorganic nitrogen species are subject to leaching from the soil columns, with NO_3 being the most mobile.

C/N ratio is a good predictor of variation in N immobilization and mineralization (van Veen et al. 1984; Aber 1992; Bradbury et al. 1993; Janssen 1996). This idea is based on the fact that heterotrophic soil bacteria usually have a lower C/N ratio than the soil they live. If it is assumed that the cells have a C/N ratio of 10 and respire about 50% of their C uptake, they may be N limited above a soil C/N ratio of 20 and C limited below (Tate, 1995). C/N ratio of soil used in the greenhouse columns was about 17 (Table 4-1). As a result, this soil was characterized as slow N immobilization and a surplus NH_4^+ was available due to synthetic graywater application and deamination of organic carbon sources.

For all sampling events except for the second one (June '10), TIN leached from graywater-irrigated columns was significantly higher than potable water-irrigated columns (Table 4-4). The higher TIN leached from the graywater-irrigated columns than that in potable water-irrigated columns was expected due to the higher input of inorganic nitrogen from the application of synthetic graywater (Table 4-3). N in the form of nitrate is very soluble and is not easily fixed to soil clay minerals, and hence, if not taken up by plants, is readily leached through drainage (Hermon et al. 2006). Candela et al. (2007) reported leaching of nitrate into deeper soil and substantially into groundwater in reclaimed wastewater irrigation. However, the risk of groundwater contamination with nitrate can be markedly controlled through appropriately matching of plant systems to effluent characteristics and using high-yielding plants (Snow et al. 1999; Rahil and Antonopoulos 2007). In this study, the grasses appeared to result in lower leaching of TIN (0.02±0.03 mg day^{-1}) compared to shrubs (0.06±0.06 mg day^{-1}; Figure 4-6). Research has indicated that dense, well-managed turfgrass areas are among the best biofiltration systems available for removal of nutrients in reclaimed wastewater (Hayes, et al. 1990; Alshammary and Qian, 2008). The lower TIN leaching from the grass columns also resulted from a greater nitrogen immobilization in the grass-soil systems. Soil OM increase was greater in graywater irrigated grass columns than the shrub columns (Table 4-7). A rapid soil OM increase likely favored N immobilization. Therefore, larger N input through graywater irrigation was retained in the soil, and TIN leaching was lower in the grass columns.

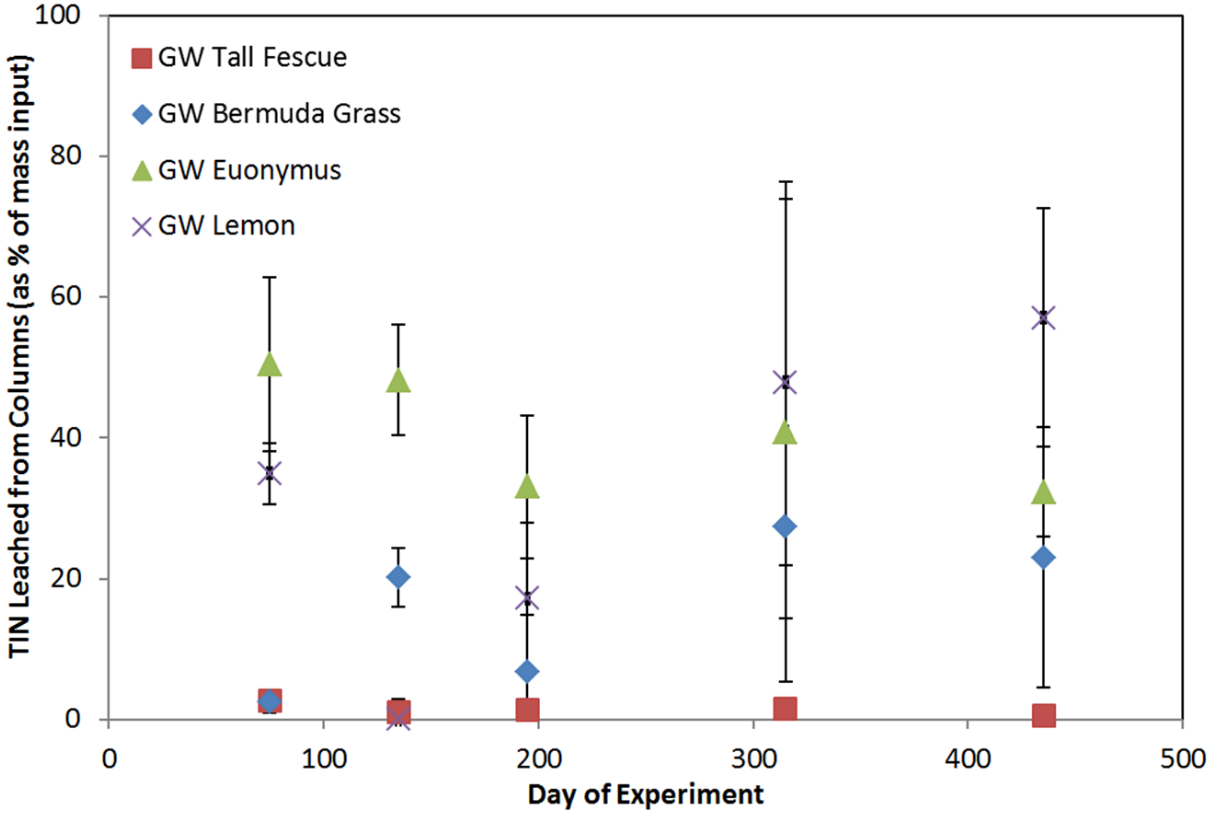

Figure 4-6. TIN Leached From Graywater-irrigated Columns. (no leachate sample was collected from lemon columns in June sampling event; GW: graywater-irrigated)

Graywater-irrigated surface soil samples had significantly higher TIN than potable water irrigated soil samples ($P \leq 0.05$; Table 4-6). While, graywater-irrigated depth soil samples (45cm) in tall fescue, Euonymus and unplanted columns had significantly higher TIN than potable water-irrigated soil samples ($P \leq 0.05$; Table 4-6), no significant difference was observed between depth soil samples collected from columns with Lemon and Bermuda grass ($P > 0.05$; Table 4-6). There was a significant decrease in TIN with sample depth (R2=0.80; $P \leq 0.05$). Surface soil samples irrigated with graywater contained 2 to 12 times more TIN than those irrigated with potable water, indicating an excess of inorganic nitrogen, which could serve as a source for observed increased biomass of graywater irrigated plants (Figure 4-3). The amount nitrogen added through graywater, which was uptaken by plants was estimated based on a mass balance for TIN to confirm the impact of graywater irrigation on plant growth. Results indicated that at the end of experiment the average expected amount of NO_3-N and NH_4-N in the soil should be 0.53, 0.37, 0.49, and 0.38 g for tall fescue, bermudagrass, Euonymus and Lemon columns respectively. However, the final TIN in the soil samples at the end of experiments showed average amounts of 0.05, 0.04, 0.22 and 0.07 g in columns with tall fescue, bermudagrass, Euonymus and Lemon columns respectively. This indicates that a large portion of NO_3-N and NH4-N was uptaken by plants during the course of experiments. This result is also consistent with observed higher plant growth in graywater-irrigated plants (Figure 4-3).

Table 4-6. Nutrients Measured in Soil Samples Collected from Different DepthsGW: graywater-irrigated; FW: potable water-irrigated; Numbers are averaged, n = 3, a, b: significantly different; a, a: not significantly different samples)

	Depth	TIN (ppm)		TP (ppm)	
Plant Type	**(cm)**	**GW**	**FW**	**GW**	**FW**
Tall Fescue	0	9.7^{a}	1.0^{b}	4.6^{a}	8.6^{b}
	25	1.4^{a}	1.4^{b}	5.4^{a}	9.7^{b}
	45	0.7^{a}	0.8^{a}	9.0^{a}	8.0^{a}
Bermuda Grass	0	5.9^{a}	2.5^{b}	8.0^{a}	10.2^{a}
	25	2.2^{a}	0.8^{b}	6.5^{a}	7.6^{a}
	45	0.9^{a}	0.8^{a}	7.0^{a}	8.0^{a}
Euonymus	0	36.9^{a}	3.0^{b}	26.8^{a}	14.3^{b}
	25	5.1^{a}	1.1^{b}	7.8^{a}	13.0^{b}
	45	8.5^{a}	1.1^{b}	13.8^{a}	13.4^{a}
Lemon	0	14.8^{a}	7.4^{b}	7.0^{a}	6.8^{a}
	25	0.7^{a}	0.9^{a}	5.8^{a}	9.0^{b}
	45	1.1^{a}	0.7^{a}	9.4^{a}	12.2^{b}
No Plant	0	25.0^{a}	3.2^{b}	23.0^{a}	8.1^{b}
	25	6.3^{a}	1.9^{b}	12.0^{a}	13.1^{a}
	45	3.9^{a}	1.8^{b}	11.0^{a}	14.0^{a}

TP was only measured in the leachate samples collected from the last two sampling events. For graywater-irrigated plants, averaged TP percentage retained in the planted columns from the last two sampling events was 79±8% and 56±5% for turfgrasses and plants respectively. No significant difference was observed in the TP leached from the graywater (0.13±0.03 mg day^{-1} and potable water-irrigated columns (0.10±0.01 mg day-1; P>0.05), indicating that phosphorus was either sorbed to soil particles or uptake by plants. Phosphorus is more readily fixed within soils than nitrogen, and therefore its transport is usually associated with plant uptake or soil transport processes such as erosion (Simard et al. 2000).

No significant difference was observed in TP measured in soil samples from graywater and potable water-irrigated plant columns except in surface soil samples collected from columns with Euonymus and unplanted columns ($P>0.05$; Table 4-6). Except in columns with Euonymus, both graywater and freshwater-irrigated soil samples had lower TP than the initial value of 14 ppm (Tables 4-1 and 4-6) despite P addition in graywater irrigated columns, thus indicating P uptake during experiments. Insignificant differences in phosphorus between graywater irrigated and freshwater irrigated soil also indicates uptake of phosphorus added by graywater. No significant increasing trend was observed for TP in leachate ($R^2=0.39$; $P>0.05$). Phosphorus likely accumulated in the surface soil of unplanted columns since there was no plant uptake (Table 4-6).

4.4.4. Organics and Surfactants

Leached DOC values ranged from 0.11 to 2.42 mg day^{-1}, far below the input values of DOC (13.6 to 19.8 mg day^{-1}; Figure 4-7). Interestingly, DOC leached from graywater-irrigated columns was not significantly different from potable water-irrigated columns except for the June '10 sampling event where DOC leached from the potable water-irrigated columns was actually higher than the potable water-irrigated columns. Given that DOC input to the graywater-irrigated columns was much higher than potable water-irrigated columns (Table 4-3), this indicates high retention of organics in the graywater-irrigated columns. While surface soil samples collected from graywater- irrigated columns had significantly higher organic matter than soil collected from potable water-irrigated columns (1.8±0.3% and 1.4±0.3%; $P\leq0.05$), no significant difference was observed for organic matter in depth soil samples collected from graywater and potable water-irrigated columns (1.0±2.0% and 1.0±0.1%; $P>0.05$; Table 4-7). Galloges (1999) reported that use of wastewater for agricultural irrigation increases the content of OM in the soil. Xu et al. (2010) observed higher total carbon in soil received reclaimed wastewater and total carbon increased with duration of application. However, Gharbi-Tarchouna et al. (2010) reported lower values of organic carbon in soil irrigated with reclaimed wastewater in comparison to soil irrigated with potable water, and attributed this trend to enhanced microbial activity resulting from more available nutrients and dissolved organic carbon.

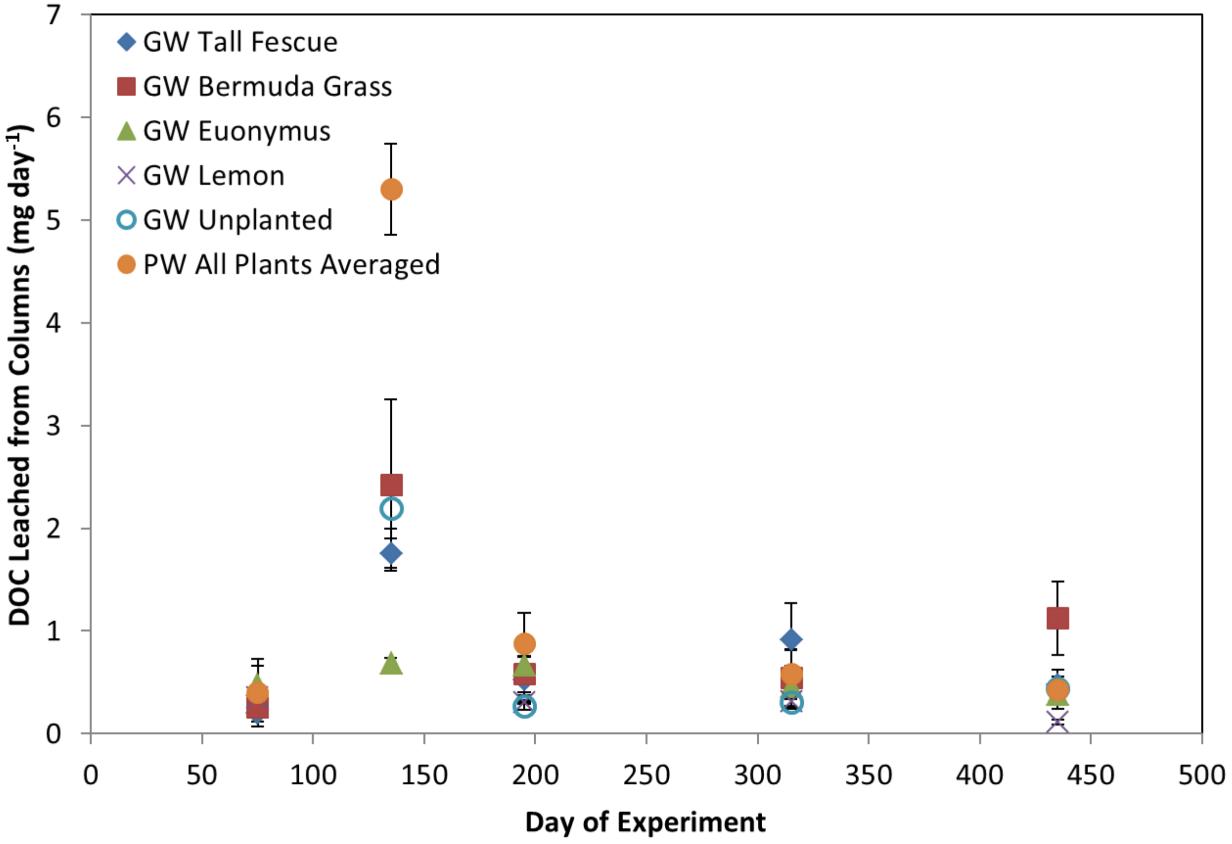

Figure 4-7. DOC Leached From Graywater-irrigated Columns. (average input DOC was 19.8, 13.6, 18.8, 14.1 and 14.9 mg day^{-1} for tall fescue, Bermuda grass, euonymus, lemon and unplanted columns respectively; no leachate sample was collected from lemon columns in June sampling event; GW: graywater-irrigated PW: potable water-irrigated)

Table 4-7. OM and Total Surfactants Measured in Soil Samples Collected from Different Depths (GW: graywater-irrigated; FW: potable water-irrigated; ND: below detection limit; a, b: significantly different; a, a: not significantly different samples)

	Depth	Surfactants* (μg kg^{-1})			OM (%)	
Plant Type	**(cm)**	**LAS**	**AES**	**AE**	**GW**	**FW**
Tall Fescue	0	460	9	6	2.0^{a}	1.9^{b}
	25	21	9	8	1.1^{a}	1.2^{a}
	45	7	3	ND	0.9^{a}	1.1^{a}
Bermuda Grass	0	768	21	10	2.7^{a}	1.7^{b}
	25	32	6	1	1.1^{a}	1.1^{a}
	45	4	3	ND	0.9^{a}	0.9^{a}
Euonymous	0	772	18	6	1.6^{a}	1.2^{b}
	25	13	2	2	1.2^{a}	1.2^{a}
	45	8	2	ND	1.2^{a}	1.1^{a}
Lemon	0	482	34	12	1.7^{a}	1.3^{b}
	25	17	5	3	1.0^{a}	0.9^{a}
	45	3	1	ND	0.9^{a}	1.0^{a}
No Plant	0	333	2	4	0.8^{a}	1.0^{a}
	25	6	6	2	0.7^{a}	0.85^{a}
	45	3	1	ND	0.8^{a}	1.1^{a}

* Surfactants were only measured in graywater-irrigated soil samples

A large component of the organic content in graywater is surfactants, and total anionic surfactants have been found to range from 4 to 34 mg L^{-1} (Wiel-Shafran et al. 2006; Gross et al. 2007; Travis et al. 2008). Leachate samples were analyzed for LAS, AES, and AE and values were summed to determine total surfactant concentration in the samples. In general, surfactants leached through the columns as a percentage of that added in irrigation water was low, 7±6% on average, for planted and unplanted columns irrigated with graywater (Figure 4-8). Results indicated that even after seventeen months of continuous irrigation with synthetic graywater, a large portion of surfactants was either adsorbed or biodegraded in the soil columns. If one were to look at the test columns as treatment system, experiment results could be interpreted as 85-98% removal through the columns. Results from conventional wastewater treatment systems indicate removal rates of LAS, AES and AE to be greater than 98% in activated sludge systems, 82-90% in trickling filters, 82-84% in a subsurface flow constructed wetland and above 87% by a wetland system consisting of a free water surface bed followed by subsurface treatment (McAvoy et al. 1999; Matthijs et al. 1999; Sima et al. 2009; Jokerst et al. 2011). Interestingly, removal of surfactant parent compounds are comparable to those achieved in wastewater treatment plants after passing graywater through only 50 cm of soil (planted or unplanted).

Of note is that average total surfactant leached from the columns increased from 2.1±0.1% to 15.2±2.7% over the duration of study (Figure 4-8). Trend analysis was conducted on average total surfactant concentration in soil samples collected from planted and unplanted columns. A significant increasing trend in average total surfactants leached from columns (planted and unplanted) over the period of the study was observed (R^2=0.86; $P \leq 0.05$). This indicates that surfactants may have more potential to leach through soil with longer duration of graywater irrigation. Of note is that the average concentrations of LAS, AES and AE in the leachate at the last sampling event (May of 2011) were 3,418±1,054, 430±160 and 169±59 µg L^{-1} respectively. Matthijs et al. (1999) reported surfactant concentrations in municipal wastewater treatment plant (MWWTP) effluent ranging from 19-71 µg L^{-1} for LAS, 2.2-13 µg L^{-1} for AES, and 1.2-12 µg L^{-1} for AE in MWWTPs using activated sludge system (Matthijs et al. 1999). McAvoy et al. (1998) reported surfactant concentrations in MWWTP effluent ranging from less than 5 up to 1,500 µg L^{-1} for LAS, 4-164 µg L^{-1} for AES, and 8-509 µg L^{-1} for AE for activated sludge or trickling filter systems (McAvoy et al. 1998). Surfactant concentration in leachate after 17 months of graywater irrigation was slightly higher than observed in treated wastewater. The potential for surfactants to migrate to groundwater warrants further study, particularly because surfactants leached from columns increased over the duration of this study.

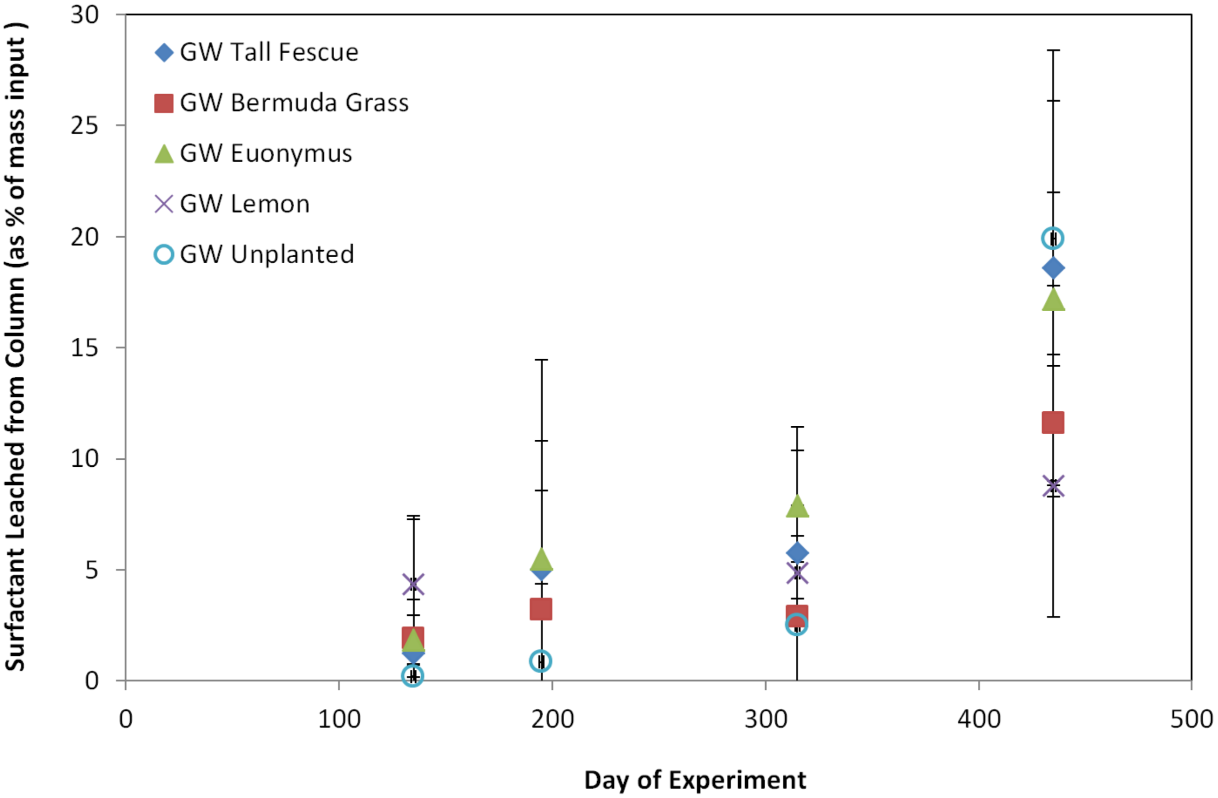

Figure 4-8. Total Surfactants Retained in the Graywater-irrigated Columns. (no leachate sample was collected from lemon columns in June sampling event)

Surface soil samples had significantly higher surfactants than deeper soil samples ($P \leq 0.05$; Table 4-7). While surface soil samples had average concentrations of LAS, AES and AE as 563±197, 17±12 and 8±3 μg kg^{-1} respectively, soil samples collected from 25 cm below the surface had average LAS, AES and AE concentrations as 18±10, 6±3, 3±3 μg kg^{-1} (Table 4-7). Concentrations were even lower in soil samples collected at a 45 cm depth. Results indicate that surfactants were adsorbed to the surface soil, which prevented transport to the deeper soil. Travis et al. (2010) reported total surfactants as 0.68±0.39, 0.15±0.06 and 0.53±0.14 mg kg^{-1}in sand, loam and loess irrigated with raw graywater respectively. Meanwhile, Negahban-Azar et al. (2012) observed average total surfactant of 0.078±0.032 and 0.030±0.025 mg kg^{-1}in graywater-irrigated and potable water-irrigated soil respectively. Surfactant concentrations measured in our study were higher, but comparable to those reported in other studies. However, it should be considered that these studies were conducted under field conditions where soil received rainwater and graywater was not continuously applied over the year.

The ratio of each surfactants reported in the synthetic graywater are averaged as 81, 17 and 3 percent for LAS, AES and AE respectively and LAS was also the dominant surfactant in soil samples (Figure 4-9). While LAS remained as the dominant surfactants in all soil samples, the relative content of AES increased with depth of soil samples (25 and 45 cm; Figure 4-9).

Results from a mass balance on surfactants indicated that 0.66-1.54, 0.04-0.08, and 0.02-0.04 mg of LAS, AES and AE respectively were accumulated in the soil columns at the end of the experiment. During the course of experiment 1619-2274, 347-487, and 69-97 mg of LAS, AES and AE were loaded to the columns through the application of synthetic graywater. During the same time, 71-175, 15-38, and 3-7 mg of LAS, AES and AE leached from the columns. Given the loaded, leached and accumulated values of measured surfactants, it can be concluded that between 92 to 96 percent of applied surfactants parent compounds were biodegraded in the soil columns. While these estimates rely on several assumptions, it is clear that a large portion of surfactants were biodegraded over 17 month duration of these experiments.

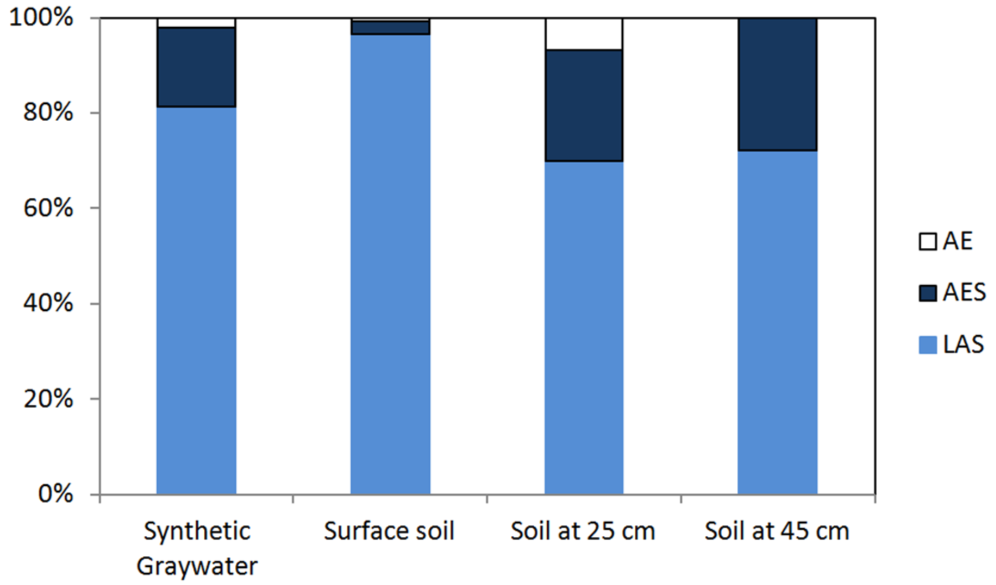

Figure 4-9. Relative Composition of Surfactants in Synthetic Graywater and Soil Samples. (percentages as molar mass)

4.5. Conclusions

The objective of this research was to determine the potential for graywater constituents to leach through soil into groundwater when graywater is applied for irrigation. The constituents with the lowest potential to leach through soil irrigated with graywater are P and B. Salts, as measured by TDS, showed potential to leach through graywater irrigated soil. A portion of the applied N was uptaken by plants, but leaching of N was still observed. Leaching of N was lower for columns planted with grass species compared to other columns. Graywater irrigation increased nutrient content (N and P) in soil, which was beneficial for plant growth. While a low percentage of surfactants added to greenhouse columns leached through, a significant increasing trend of surfactants leached from columns was observed over the 17 month duration of the study ($R^2=0.86$; $P\leq 0.05$). More research is required to determine if leaching of surfactants would continue to increase over time. A mass balance on surfactants showed that 92-96% of added surfactant parent compounds were biodegraded.

Chapter 5

FATE AND TRANSPORT OF LINEAR ALKIYLBENZENE SULFONATES, ALKYL SULFATES, ALKYL ETHOXYSULFATES, AND ALCOHOL ETHOXYLATES IN VARYING SOIL TYPES[1]

5.1. Introduction

A large component of the organic compounds in graywater is surfactants. Surfactants are used in household cleaning products, cosmetics, detergents, lubricants (and other miscellaneous industrial applications). Surfactants present in graywater are of concern due to their potential toxicity on plants and soil organisms, effects on soil physical property and their effects on mobility of hydrophobic compounds in the soil. In addition, surfactants applied in graywater may be transported to groundwater. Surfactants have been identified as components of graywater that can cause water repellency and reduce soil hydraulic conductivity (Lado and Ben-Hur, 2009; Travis et al. 2008; Wiel-Shafran et al. 2006). In addition, surfactants may affect the mobility and degradation of hydrophobic organic compounds in soil or sediment (Edwards et al. 1994; Tiehm 1994). Research on fate and transport of surfactants in soil irrigated with graywater is limited. Some recent studies on graywater indicated accumulation of surfactants in soil after graywater application (Wiel-Shafran et al. 2006; Travis et al. 2010). In field studies, graywater was determined to significantly impact surfactant concentration in soil. Total surfactants measured at households with systems in place for more than five years and households with newly installed systems were 219±79 µmol kg^{-1} and 486±130 µmol kg^{-1} respectively (Chapters 2 and 3).

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[1] This phase of the project is ongoing as a M.Sc. thesis by Zhaohua Huang.

One of the main mechanisms responsible for surfactants mobility is sorption. It is expected that surfactants will adsorb to soil particles due to their intensive surface activity and hydrophobic characteristic (Ying 2006 and Boluda-Botella et al. 2010). However, the sorption of surfactants (e.g. LAS) was reversible according to data reported by Boluda-Botella et al. 2010. As a result, anionic surfactants may reach deeper soil if sufficient water is applied to the soil.

The main processes responsible for surfactants interaction with soil are biodegradation, sorption and precipitation (Boluda-Botella et al. 2010). Due to intense surface activity and hydrophobic characteristics of anionic surfactants (LAS and AS/AES) and nonionic surfactants (AE), sorption is one of the most relevant processes in their transport through the soil. Boluda-Botella et al. (2010) investigated the fate of LAS in agricultural soil. The researchers found that LAS sorption was the most relevant process in LAS transport through soil and it was stronger in agricultural soil with higher organic content than sand, especially for longer chain homologues. In addition, it was found that lower chain homologues could access deeper soil layers more quickly with sufficient water volume. In their study, the biodegradation of LAS reached 25% of the total input in continuous flow, limiting LAS accumulation substantially. Another study by van Compernolle et al. (2006) showed that AE can adsorb strongly onto solids (log K_d ranging from 1.6 to 4.9), thereby reducing bioavailability of AE in the environment. However Droge and Hermens (2010) found that the presence of co-solutes strongly affects the sorption isotherms of individual compounds, when (i) mixtures of surfactants which compete for the same adsorption sites are used and (ii) adsorption sites become saturated. In graywater, different surfactants and other chemical constituents exist, and as a result, the sorption behavior of individual surfactants may vary from when they are present individually. However, the low concentration of surfactants may reduce intensity of sorption competition. This theory has not been investigated and was assessed during the column study. During the column studies, surfactants were applied within the range of their concentration found in graywater. This concentration was lower than the concentration used in most of the mobility studies, and it enabled investigation of hypotheses under SO2 and SO3 (Section 1-8). Consequently, during the column studies, the main processes responsible for fate and transport of surfactants, effects of soil characteristics on surfactants mobility, and leaching organic matters from soil columns were evaluated addressing SO2, SO3, and SO4 (Section 1-8).. Three types of soil were studied to investigate the effect of soil composition on fate and transport of surfactants. These three soil types were native sandy loam, native sandy loam with compost and native sandy loam mixed with clay soil respectively.

Experiments with the sandy loam columns and sandy loam columns with compost have been conducted and preliminary results have been demonstrated in the following sections. Of note is the last part of this experimental study with sandy loam mixed with clay soil columns is ongoing and will be followed up by a M.S. student in our research group as part of her thesis. I have contributed to experiment setup and data collection for the initial experiments.

5.2. Experimental Procedure

5.2.1. Column Setup

A column test was conducted where fate and transport of surfactants through soil was studied. A native sandy loam soil was obtained from Pioneer Sand Company (Fort Collins, CO.) and used for the experiments. The soil composition was 65% sand, 17% silt and 18% clay. Synthetic graywater was applied to the columns and the leachate was collected. Leachate samples were analyzed for surfactants and TOC. After the leaching process, soil was removed from the columns and sectioned into an appropriate number of segments. Each soil segment was then analyzed for surfactants. In addition, a tracer test was conducted on soil columns before initiation of the tests and at the end of the study respectively.

A clayey soil from CSU research farm was added to the soil to achieve varying clay content. Three columns were prepared for each soil type including greenhouse loam soil, the sandy loam soil with addition of 3% compost and the sandy loam soil with addition of 50% clay soil. Soil characteristics are shown in Table 5-1. These soil types provided data on the effect of organic matter and clay content on fate and transport of surfactants. Results are not yet available for the 50% clay content columns (C).

Table 5-1. Soil Characteristics

Soil	Sand (%)	Silt (%)	Clay (%)	pH	EC (µS cm^{-1})	OM (%)	TN (%)	SAR
S	50	17	33	7.4	1150	0.65	0.09	3.3
O	46	16	38	7.4	1400	1.1	0.13	3.7

S: native sandy loam
O: native sandy loam + 3% organic compost

Experiments were conducted under abiotic conditions (Table 5-2). To provide the abiotic condition in the columns, soil was autoclave at 215^{o}C for 30 minutes three times in three consecutive days exactly before packing into columns. In addition 0.5% formaldehyde was added to the synthetic graywater to prevent microbial growth. This approach has been used before by other researchers (Boluda-Botella et al. 2010). It should be noted that adding formaldehyde to the synthetic gray water increased the TOC from 90-100 mg L^{-1} to 2100-2200 mg L^{-1}. The total number of columns was twelve including one control column for each set. Column tests were performed based on ASTM standard D4874 – 95 and using a method described by Boluda-Botella et al (2010).

Table 5-2. Columns setup

Test	Porous Medium	Formaldehyde
S	100% Sandy Loam soil	0.5%
O	97% Sandy Loam soil + 3% compost	0.5%
C	50% Clay soil + 50% Sandy Loam soil	0.5%

PVC columns (45 cm in length and 12 cm internal diameter) were used for the experiments (Figure 5-1). All experiments were conducted at room temperature (20 - 25 °C) and under saturated condition. Air dried and sieved soil with 100 mm sieve was packed and compacted in each column in two stages by tapping repeatedly until a soil depth of 30 cm is achieved during each stage according to ASTM standard D4874 – 95. After packing, bulk density was measured for each column.

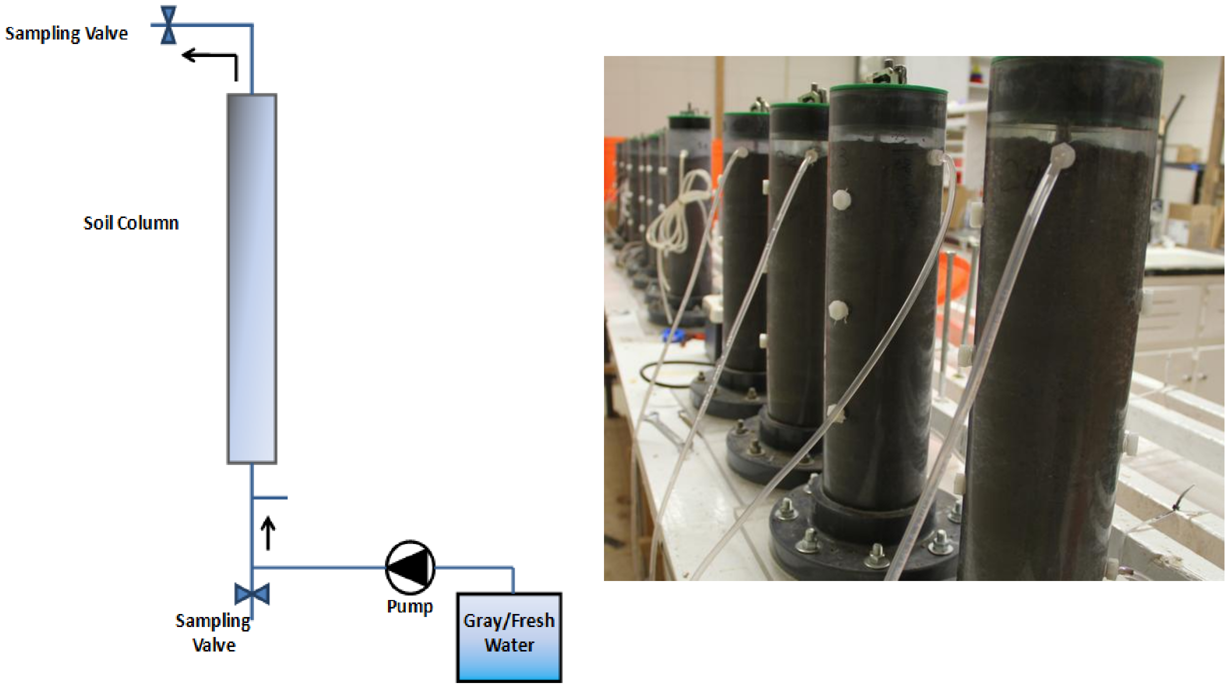

Figure 5-1. Schematic of column set up

5.2.2. Synthetic Graywater

The synthetic graywater (Table 5-3) was formulated to contain constituents typically found in graywater sources determined during the field experiments (chapter 3, section 4.3) and by others (Gerba et al 1995, Christova-Boal et al 1996, Finley et al 1998, Friedler 2004, Ramon et al 2004, Gross et al 2005, Shafran et al 2006, Roesner et al 2006, Pidou et al 2008). Synthetic graywater was composed in D.I. water the same day which was planned to apply for irrigation in order to avoid any changes in composition. Yeast extract was included in the synthetic graywater solution to simulate organics in graywater of which the source is not surfactants. The synthetic graywater contained 35 mg L^{-1} of total surfactants (15, 16, 4 mg L^{-1} of LAS, AS/AES, and AE respectively). Yeast extract is commonly applied to simulate organic matter in graywater (Fenner and Komvuschara 2005; Panikkar et al 2010).

Table 5-3. Synthetic Graywater Recipe

Reagent	Concentration (mg L^{-1})
Ammonium Chloride Crystalline	8.5
Sodium Nitrate	15.8
Sodium Borate	4.4
Potassium Phosphate monobasic	3.5
Magnesium Sulfate Anhydrous Powder	57.3
Potassium Chloride Crystalline	11.4
Calcium Chloride	47.1
Sodium Chloride	25.6
Calcium Sulfate	143.3
Sodium Sulfate	40.5
LAS (C_{12})	15.0
AS	4.0
AES (EO_1)	4.0
AES (EO_2)	4.0
AES (EO_3)	4.0
AE	4.0
Yeast Extract	248.3

5.2.3. Irrigation and Sampling Procedure

Experiments were conducted under saturated condition. Each column was conditioned with D.I. until soil reached equilibrium, defined here as reaching constant EC in the leachate with no more than 5% variation observed over three data points and constant flow in the inlet and outlet. Synthetic graywater was applied in to the columns as a 4 h pulse feed at a constant flow rate of 5 ml min^{-1}. Homogeneity of surfactants solution was maintained with adequate stirring during the experiment. Consequently, approximately 1.2 L of synthetic graywater was fed into columns during this part of the experiment. This amount was approximately equal to irrigation volumes used at the greenhouse study (Section 4-2). After 4 hours of irrigation with graywater, the inlet flow was changed to D.I. water with the same flow rate and continued for about 70 hours. Based on other research, it was expected that most of the surfactants leaches from the columns in 70 hours. During the sampling events, leachate samples were collected in 50 ml dark glass sampling bottles. Total number of 10 leachate samples was collected from each column setup and samples were kept in the refrigerator during the experiments. The first leachate sample was collected at 0.5 hour after starting the experiment, next three samples were collected at 4 hours intervals and the next 6 sample were collected at 10 hours intervals (Table 5-4). Leachate samples were extracted and analyzed for surfactants and TOC immediately after finishing the experiments according to the methods described in section 3.3.

Soil samples were collected at the end of experiment from all of the columns at three different depths of 15, 30 and 40 cm from the inlet. Approximately 20 g of soil was collected at each sampling point in 50 ml centrifuge tubes. Soil samples were immediately extracted after collection for surfactant analysis according to methods described in section 2.3.

Table 5-4. Leachate Sampling Intervals

Sample No.	Sampling Time (h)
1	0.5
2	3.5
3	7
4	10.5
5	20.5
6	30.5
7	40.5
8	50.5
9	60.5
10	70.5

5.2.4. Tracer Test

Before starting the experimental study and after finishing the experiments, the hydrodynamic parameters of soil columns were characterized by conducting a tracer test. Bromide, chloride and fluorone dyes are the most commonly used tracers in soil column studies. In this research, chloride as $CaCl_2$ was used as tracer. According to Sardin (1998) $CaCl_2$ is considered a good tracer in experiments with sand columns and clay. Tracer test was conducted under saturated condition. First sufficient amount of CaCl2 solution (15 to 20 L) was prepared in D.I. water which had the electrical conductivity of 2000 µS cm^{-1}. Tracer solution was added to the columns at continuous flow rate of 5 ml min^{-1} and EC was continuously measured in the effluent every 15 to 30 minutes. The tracer test was terminated when EC in the effluent reached a constant value for three consecutive measurements. Breakthrough curves were constructed based on the data obtained from the tracer test. The obtained breakthrough curves were used to determine column hydrodynamic characteristics (Boluda-Botella et al. 2007 and 2008). The software ACUAINTRUSION (Boluda-Botella et al. 2006) was used for tracer test data analysis.

This program provided the calculated transport parameters for the soil columns. These parameters included residence time, Darcy velocity, porosity, interstitial velocity, dispersion and dispersivity.

The ACUAINTRUSION interface calculates the best fit from tracer test data according to the analytical solution of the convection-dispersion equation (Equation 5-1; Lapidus and Amundson, 1952).

Equation 5-1.

$$C(L,t) = C_i + \frac{(C_0 - C_i)}{2}\left[\operatorname{erfc}\left(\frac{L - vt}{\sqrt{4D_L t}}\right) + \exp\left(\frac{vL}{D_L}\right)\operatorname{erfc}\left(\frac{L + vt}{\sqrt{4D_L t}}\right)\right]$$

Where (for example, for the Cl^- experimental data): C (L, t), is the Cl^- concentration at the output stream of the column; C_i, the initial Cl^- concentration in the water; C_0, the concentration of chloride at the inlet; L, column length; t, time; v, interstitial water velocity in the direction of propagation (equal to Darcy velocity divided by porosity); and DL, the longitudinal dispersion coefficient.

This program provides various transport parameters (Tables 5-2 and 5-3) including mean residence time tm (L/v), Péclet number, (Pe = v_L/D_L), effective porosity e, interstitial velocity v(u/e), D_L, and dispersivity α(L/Pe).

5.3. Results and Discussion

Of note is results presented in the following sections are preliminary and further analysis is required.

5.3.1. Tracer Tests

Physical and hydrodynamic characteristics of all soil columns were determined at the beginning and at the end of experiments. A continuous inflow tracer test with $CaCl_2$ was used to construct breakthrough curves based on conductivity of effluent samples which were then used to estimate hydrodynamic and physical properties of soil columns (Figures 5-2 and 5-3). The determination of the transport parameters is relevant to adequately explain surfactants behavior in columns packed with different soil (Boluda-Botella et al. 2010).

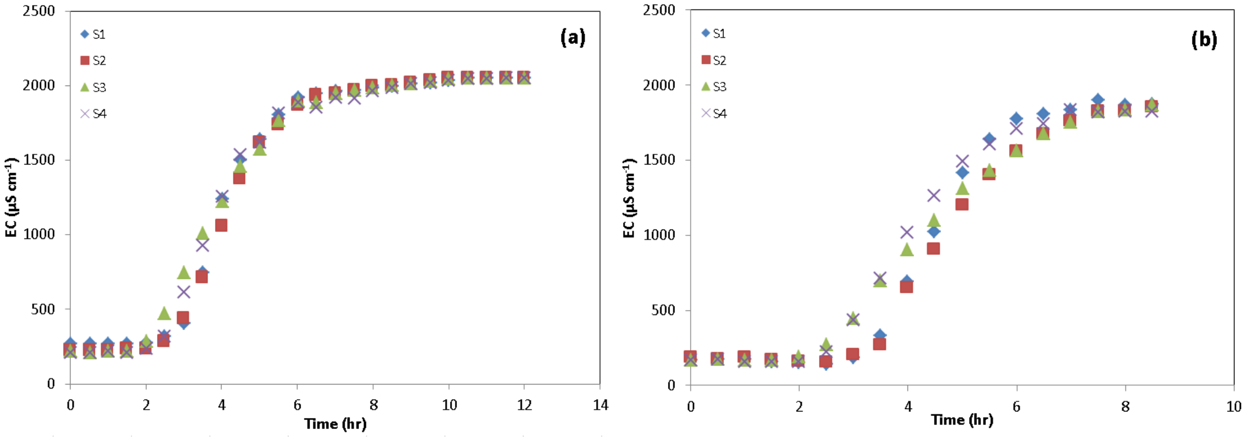

Figure 5-2. Tracer Tests Result for Sandy Loam Columns. (S1 to S3: graywater-irrigated columns, S4: control column; a: before experiment b: after experiment)

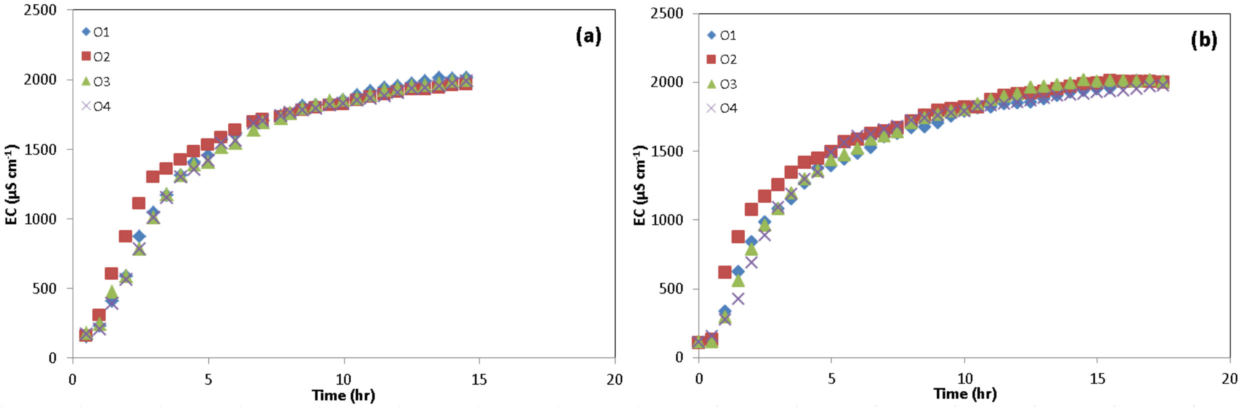

Figure 5-3. Tracer Tests Result for Sandy Loam Columns with Compost. (O1 to O3: graywater-irrigated columns, O4: control column; a: before experiment b: after experiment)

The flow through the soil column was similar for three columns prepared in parallel at the same time (sandy loams and sandy loams+compost), and indeed for experiments developed at different times. Consequently, it can be concluded that the main flow properties for the different tests were repeatable and reproducible.

Mean residence times were 4.33±0.27 and 4.12±0.45 h in sandy loam and sandy loam with compost columns respectively. Slightly lower residence time in sandy loam with compost columns was due to lower effective porosity of soil columns because of compost addition. Addition of compost into the soil columns decreased the effective porosity from 0.38±0.2 in sandy loam columns to 0.37±0.03 in sandy loam with compost columns. The small dispersivities (< 0.10 and <0.40 cm in S and O columns respectively) indicated that the column hydrodynamic characteristics were similar to plug flow.

5.3.2. Surfactants Behavior in Soil Columns

5.3.2.1.Leaching of Surfactants through Soil Columns

Due to their chemical features, surfactant molecules may sorb directly onto solid surfaces or may interact with sorbed surfactant molecules. The sorption mechanism is dependent on the nature of the sorbent and the surfactant concentration (Adeel and Luthy 1995; Brownawell et al. 1997; Fytianos et al. 1998; Ou et al. 1996). Log K_{ow} is a good indicator to determine and compare hydrophobicity of different compounds. Among the surfactants studied in this research, AES has the lowest Log K_{ow}, hence lowest hydrophobicity. Average Log K_{ow} is 3.32, 0.1 and 5.36 for LAS, AES and AE respectively (HERA 2002, 2003, 2009a and 200b). In the both soil types, the variation of surfactants eluted from soil columns has been demonstrated in Figures 5-4 and 5-5. Surfactants that access first, and in greater extent to the deeper soil layers are those with less hydrophobicity such as AES. However, the masses of AE and LAS were low in the first samples due to their higher hydrophobic characteristics which resulted in higher sorption capacity on soil particles. In sandy loam with compost columns AES, LAS and AE required >4, >10 and >50 h to start leaching from the soil respectively (Figures 5-4 and 5-5). In sandy loam columns with no compost, AES and LAS required >10 and >20 h to start leaching from the column respectively. It was concluded that adding compost to the soil decreased the retention of the columns and increased percolation rate of water trough columns.

The behavior of surfactants in soil can be determined by a competition between biodegradation, sorption and leaching (Narkis and Ben-David 1985; Kuchler and Schnaak 1997; Ou et al. 1996; Abu-Zreig et al. 1999; Yuan and Jafvert 1997; Boluda-Botella et al. 2010). In the soil columns studied in this research, because of the abiotic condition, effect of biodegradation was negligible. As a result, soil column acted as a reverse chromatographic column (Figures 5-4 and 5-5). The surfactants mixture was separated during transport, since the retardation factor in relation to the tracer was higher for longer alkyl chain-length homologues, as previously found in field experiments (Krueger et al. 1998a,b). It is relevant that adding compost to the soil decreased the retention time of the soil columns which caused faster leaching of surfactants in the columns with compost. It increased the drainage capacity of soil and water percolated faster in the columns with compost. However, results showed that less amount of surfactants leached from sandy loam columns with compost than sandy loam columns. Results confirmed that adding organic matters to the soil increased adsorption of surfactants and decreased their leaching rate (Figure 5-4 and 5-5).

Table 5-5. Sorption characteristics (HERA 2002, 2003, 2009a,b and references there in)

Surfactants	K_d	Log K_{ow}
LAS	2-300	3.32
AES	-	0.1
AE	580-5900	5.36

Sorption characteristics of LAS, AES and AE are summarized in Table 5-5. Log Kow is a good indicator of to evaluate hydrophobicity of organic compounds. The relative Log K_{ow} of surfactants studied here can be summarized as AE>LAS>AE (HERA 2002, 2003, 2009a and 200b). As a result AES had the highest leaching potential compare to AE and LAS. This result is consistent with what we observed in greenhouse experiments. Results from the greenhouse experiments showed higher AES in the deeper soil which shows higher leaching potential for AES compared to LAS and AE (section 4.5.4).

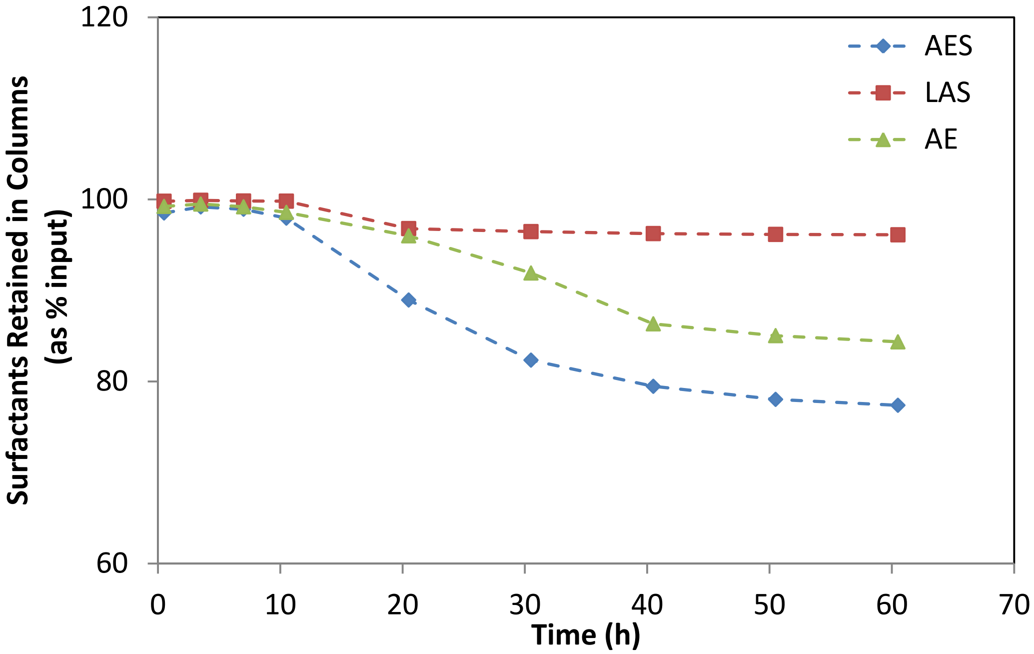

Figure 5-4. Surfactants Retained in the Sandy Loam Columns as percentage of Input (n=3)

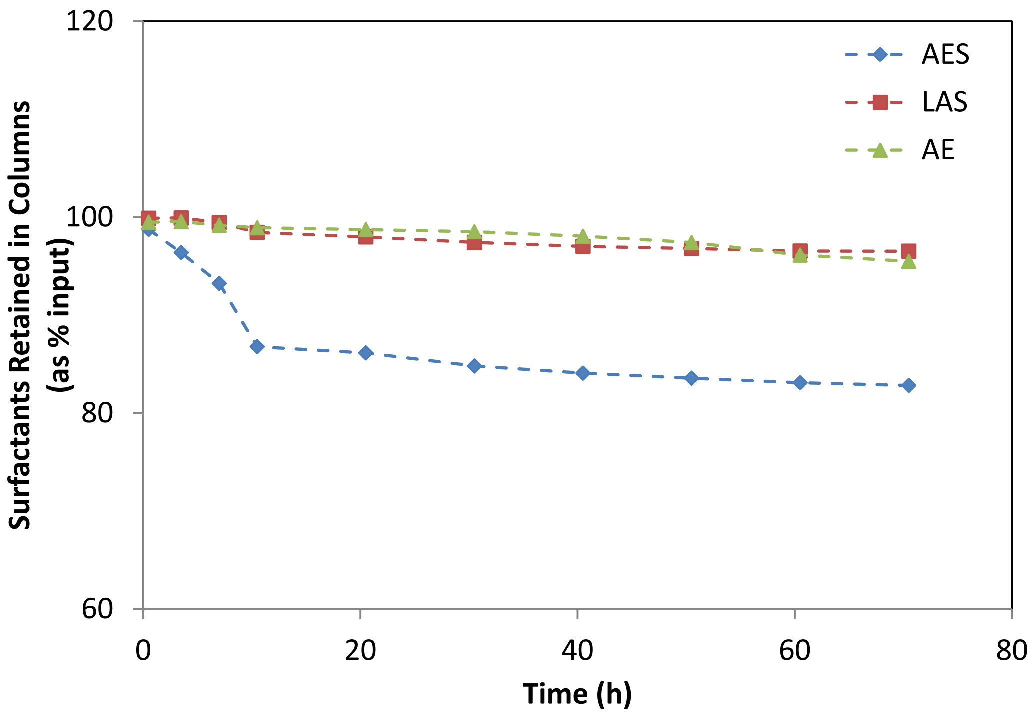

Figure 5-5. Surfactants Retained in the Sandy Loam Columns with Compost as Percentage of Input (n=3)

In sandy loam columns with compost total mass of surfactants leached from columns during the course of experiments were 6.94±1.66, 1.31±0.75 and 0.46±0.11 mg for AES, LAS and AE respectively. In sandy loam columns with no compost, total mass of surfactants leached from columns during the course of experiment were 8.72±2.67, 1.41±0.74 and 1.51±0.51 mg for AES, LAS and AE respectively. It was observed that while approximately 43, 9 and 11% of added AES, LAS and AE leached from sandy loam columns with compost, 55, 10 and 38% of added surfactants leached from sandy loam columns with no compost. Results indicated that adding compost resulted in more retention of surfactants in the soil columns more likely by increasing OM content of the soil, which provided more adsorption sites. Consequently, adding

3% compost increased OM of the soil columns 90% (Table 5-1). This increase in OM resulted in 20, 7 and 70% increase in retaining of surfactants in the soil columns.

Surfactant concentration varied in soil samples collected from different depths of the sandy loam columns with compost (Figure 5-6). Results obtained from soil analysis revealed that more LAS were adsorbed to the soil than AE. Results also indicated that LAS was transported through the soil columns and detected in considerable amounts after leaching through 40 cm of soil.

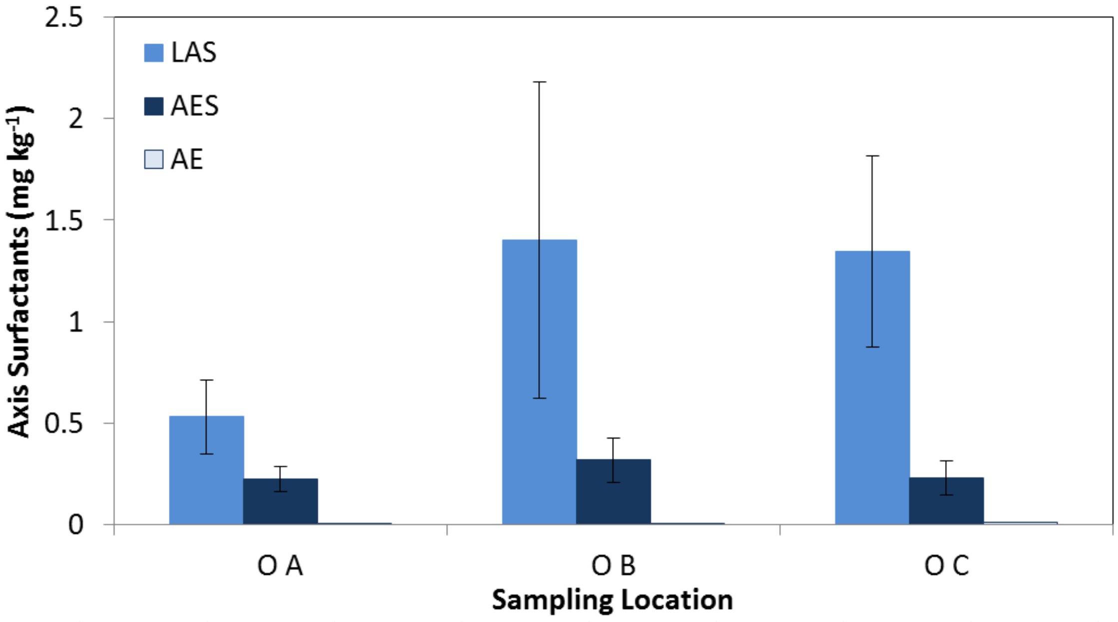

Figure 5-6. Variation of Surfactant Concentration in Soil Samples (O: sandy loam with compost; A: 15, B: 30 and C: 40 cm from the inlet)

5.3.2.2.Leaching of AES homologues through Soil Columns

The variation of AES homologues retained in the soil columns are shown in Figures 5-7 and 5-8. AES homologues that leached first and to the greater extent are those with a lower number of EO groups (AS and AES EO1) and smaller molecular weights. However, AES-EO2 and AES-EO3 leached slower from the columns due to their stronger interaction with soil particles. While there are many researches on fate and transport of LAS and AE in soil, limited data is available on fate and transport of AES in soil (Narkis and Ben-David 1985; Kuchler and Schnaak 1997; Ou et al. 1996; Abu-Zreig et al. 1999; Yuan and Jafvert 1997; Fytianos et al. 1998; Boluda-Botella et al. 2010). Different studies showed that moving ability of alcohol ethoxylates (AE) in soil decreased when number of EO group increased (Narkis and Ben-David 1985; Yuan and Jafvert 1997). The same phenomena was observed in this study where AES with no or lower EO groups leached faster from the columns than AES with higher EO (Figures 5-7 and 5-8).

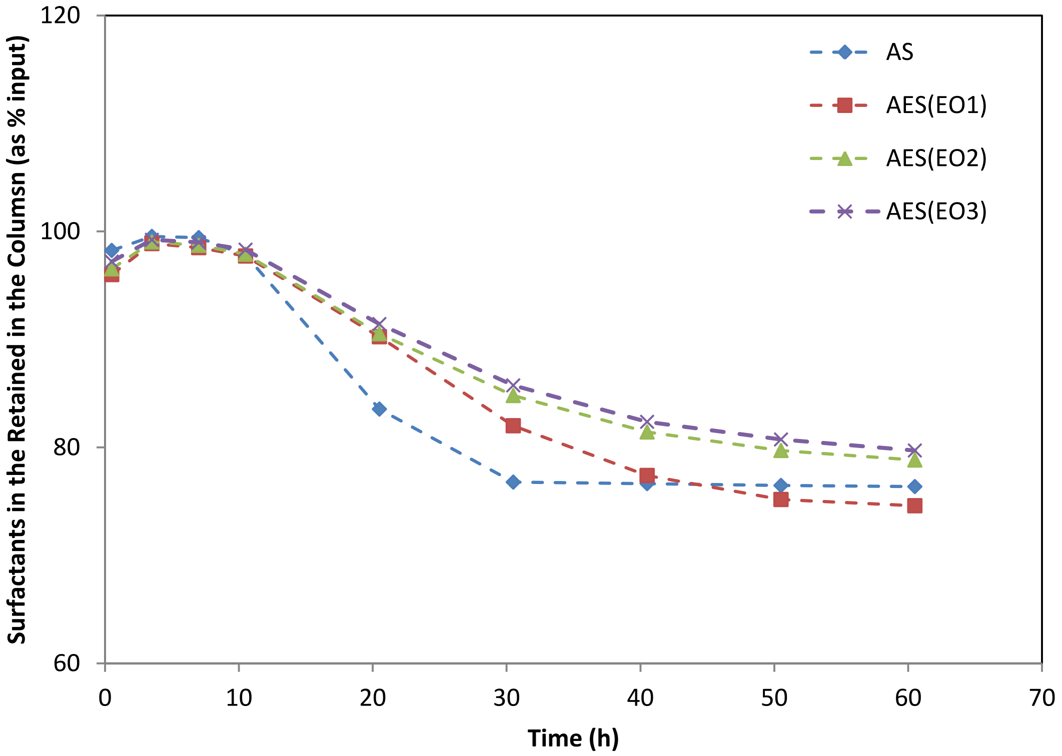

Figure 5-7. AES Homologue Retained in the Sandy Loam Columns as Percentage Input (n=3)

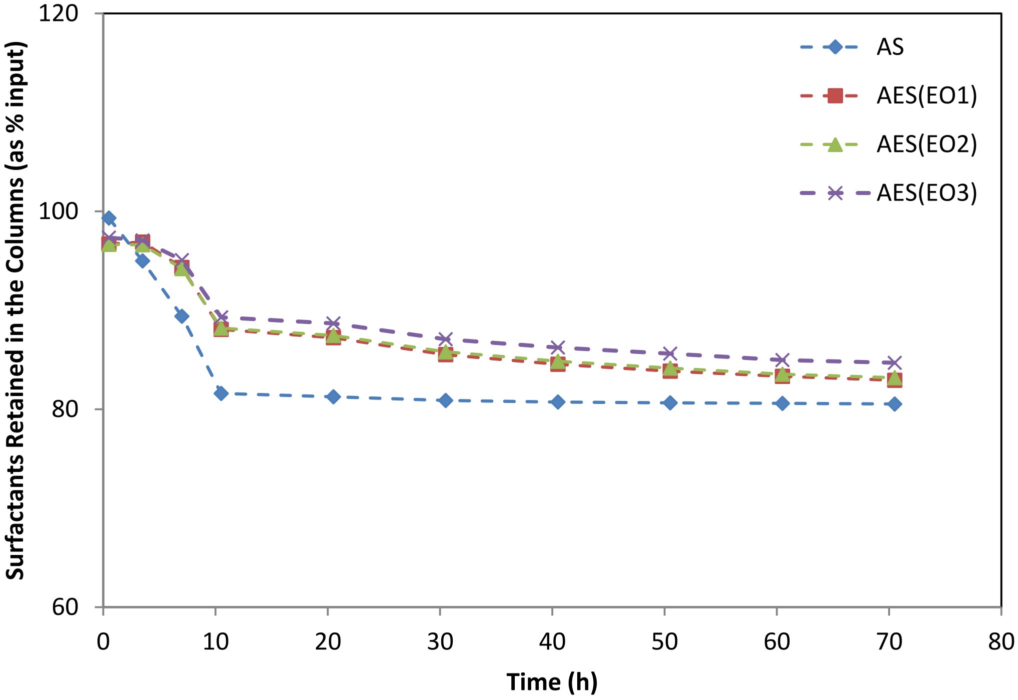

Figure 5-8. AES Homologue Retained in the Sandy Loam Columns with Compost as Percentage Input (n=3)

Soil columns acted as a reverse chromatographic column where AES homologues were separated based on their molecular weight and number of EO groups (Figure 5-9). Soil analysis revealed that while the initial concentration of AE homologues were equal in the synthetic graywater, homologues with higher molecular weights and number of EO groups adsorbed more to the soil than homologues with lower EO groups. Results also indicated that AES-EO3 had the lowest mobility among the AES homologues measured in this study as more AES-EO3 was detected in the sampling location close to the inlet (Figure 5-9). These results were consistent with concentration gradients in the leachate which showed lower leaching rate for AES-EO2 and EO3 (Figures 5-7 and 5-8). Results were consistent with the greenhouse study as AES with lower EO was detected more in the deeper soil (section 4.5).

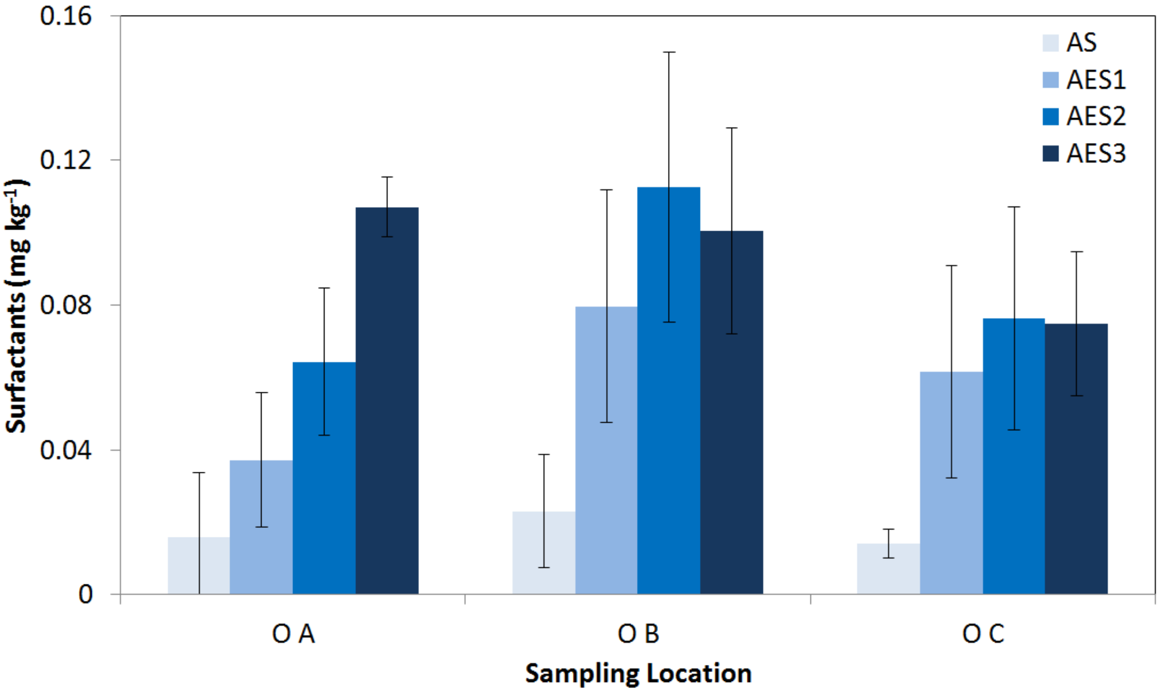

Figure 5-9. AES Homologue Concentration in Soil Samples (O: sandy loam with compost; A: 15, B: 30, C: 40 cm from the inlet)

5.4. Conclusion

This study investigated sorption of surfactants in the absence of biodegradation through different soil columns. Two types of soil were used in this study including sandy loam and sandy loam with compost. Results indicated that while amending the soil with compost decreased the retention time of surfactants in the soil columns, less surfactants leached from the columns with higher OM. Consequently, adding 3% compost increased OM of the soil columns 90%. This increase in OM resulted in 20, 7 and 70% increase in retaining of surfactants in the soil columns. Sorption is one of the most relevant processes in the surfactants transport through the saturated soil zone due to the intense surface activity and hydrophobic character of surfactants. Among the surfactants measured in this study, AES had the highest mobility compared to LAS and AE. Results from this study indicated that in the absence of biodegradation which usually occurs when soil is saturated and anaerobic, sorption is the main process which controls the fate and transport of surfactants. In case of concern associated with groundwater contamination, using surfactants with higher EO groups may decrease the mobility and leaching potential of surfactants. Consistent with our results from field and greenhouse study, AE is the most favorable surfactants compared to LAS and AES in terms of less leachability to the deeper soil. Since AE has the highest sorption capacity and it is readily biodegradable, it is not likely leach to the deep soil or transport to groundwater. Result also indicated that AE was more affected by changes in OM of the soil compared to LAS and AE.

AES retention was stronger in sandy loam soil, particularly for longer-chain homologues than sandy loam soil with compost. Consequently, AS and AES-EO1 could access deeper soil layers more quickly. However, AES-EO2 and especially AES-EO3 required high volumes of eluted water to be transported through the soil columns. Results indicated that if adequate amount of water is being applied to the soil, surfactants start to transport to deep soil to some extent. Therefore, in case of excessive irrigation, leaching of surfactants can be a concern.

Chapter 6

SUMMARY AND CONCLUSIONS

The objective of this research project was to elucidate information on effects of graywater irrigation on soil quality and the fate and transport of surfactants in soil as a result of its application for residential landscape irrigation. Experimental studies were conducted in three parts: field studies, greenhouse experiments and column studies. First phase of the project was field studies which included households with existing systems and households with newly installed systems. Second phase of the research was, a greenhouse experiment, which was conducted to evaluate leaching potential of graywater constituents in soil-plant systems. The last part of the research was a column study that focused on fate and transport of surfactants in soil columns. Results from all three phases of the project are summarized in the following sections.

6.1. Effects of Graywater Irrigation on Soil Quality and Leaching Potential of Constituents

6.1.1. Salts

Sodium accumulation has been a problem for reclaimed water irrigation and is also a concern for graywater irrigation. In the field studies, while sodium does appear to accumulate in areas irrigated with graywater, as observed by increased SAR and EC at some sampling locations, this accumulation is not in the range of high concern for plant health and soil quality. SAR was always below 5 in soil samples collected from field studies (Chapters 2 and 3), low enough to prevent any harmful effect for plants water uptake. However, greenhouse studies indicated a potential for salts in graywater to leach through soil, potentially migrating to groundwater.

While B accumulation was not observed in graywater-irrigated areas at households with newly installed systems over two years of monitoring, elevated B was observed at the household in TX where graywater was applied for irrigation for 31 years. In the greenhouse study, B was significantly higher in soil in graywater-irrigated pots compared to potable water-irrigated pots. However, in the greenhouse study B was still below the deteriorative level for plant growth of 5 mg kg^{-1} in all soil samples. Overall, results do indicate a potential for B accumulation in soil when applied in graywater for irrigation. In addition, B has potential to leach to groundwater, but there is not a human health risk posed from B in groundwater.

6.1.2. Nutrients

In general, field results did not indicate significant differences in nutrient content of soil when graywater was applied for irrigation. AZ and CO households with new graywater systems were the only households where soil NO_3-N levels were significantly elevated under graywater irrigation over time. In the greenhouse experiments, graywater-irrigated soil had higher TN values than potable water-irrigated soil, indicating additional nitrogen available in soils irrigated with graywater. In this study, TN leached from graywater irrigated columns as a percentage of mass added, ranged from 20-80%. In addition, TN measured in leachate from graywater irrigated columns was higher than potable water irrigated columns. While some nitrogen added from graywater is likely assimilated by plants, there is still potential for nitrogen to leach through soil and to groundwater especially in the form of NO_3. Phosphorus did not accumulate in soil samples collected in the field study or the greenhouse study, and no significant difference was observed in phosphorus leached from graywater and potable water-irrigated columns.

6.1.3. Organic Matters

OM was sometimes elevated in graywater-irrigated soil samples collected from the field study. Of the homes with new graywater system installations, AZ and CO graywater-irrigated surface soil contained 20-50% and 35-53% more OM compared to soil received freshwater respectively. At the Texas household where graywater was applied for irrigation for more than 31 years, OM was notably higher in graywater-irrigated soil compared to freshwater-irrigated soil. Results from the greenhouse study indicated an impact of graywater irrigation on OM in surface soil. An increase in OM is considered beneficial for both soil quality and plant health. Results from the greenhouse experiments indicated that surface soil samples irrigated with graywater had significantly higher OM than potable water-irrigated soil samples. However no difference between organics leached from graywater and potable water-irrigated columns was observed. It can be concluded that graywater irrigation was beneficial by increasing the organics in the surface soil and no concerns was associated with leaching of organics into deeper soil and potentially ground water.

6.1.4. Infiltration

Theoretically, graywater decreases the infiltration rate of water into soil in unsaturated zone because of the reduction in capillarity due to surfactants present in graywater. However, data obtained from field studies was too variable to make strong conclusions on the impact of graywater irrigation on infiltration. While at some of households, observed infiltration rates were higher in areas irrigated with graywater compared to freshwater, at some others, observed infiltration was higher in areas irrigated with freshwater. Infiltration tests were also conducted on planted columns and columns with no plant in the greenhouse study. In the greenhouse experiments, infiltration rates were mostly higher in graywater-irrigated columns and the difference was statistically significant in unplanted columns. Based on combined results from the field and greenhouse studies, it can be concluded that if graywater is applied directly with hose application or bucket, no negative impact will be observed because movement of water in soil is controlled more by gravitational flow. However, drip irrigation raises the negative impacts of graywater irrigation on infiltration rate since in this case capillary flow is also a main mechanism that controls the flow of water in soil.

6.1.5. Antimicrobials

Graywater irrigation caused accumulation of antimicrobials in soil as antimicrobials were detected in surface soil samples irrigated with graywater. Even though antimicrobials were only detected in surface soil samples (0-15 cm), the concentration of TCS (3.8-6.3 mg kg^{-1}) and TCC (2.8-9.1 mg kg^{-1}) were notable in those areas where detected. TCS was higher than has been observed in biosolids amended soil (Cha and Cupples; 2009). A concern associated with high concentrations of antimicrobials in soil would be decreased microbial activity. Further investigation is warranted to determine the effect of graywater irrigation on antimicrobial concentration in soil and the impact this may have to soil microbiology and the potential formation of antibiotic resistant genes.

6.2. Fate and Transport of Surfactants

6.2.1. Accumulation

In field studies, graywater was determined to significantly impact surfactant concentration in soil as graywater-irrigated soil samples had significantly higher surfactants than freshwater-irrigated soil samples at all sampling locations. Interestingly, at all three households, where a new graywater irrigation system was installed, surfactants in soil increased from the baseline sampling event then remained fairly constant over time. Some minor variation was noted where concentration was higher at the end of the dry season compared to the wet season. In addition, total surfactants measured at households with systems in place for more than five years and households with newly installed systems were comparable (219±79 µmol kg^{-1} and 486±130 µmol kg^{-1} respectively). Surfactant concentration in soil collected from the greenhouse experiment was higher, ranging from 940 to 2212 µmol kg^{-1}, likely a result of lack of rainfall in the greenhouse experiments. While it is clear that graywater irrigation results in accumulation of surfactants in soil, there is no evidence that accumulated surfactants have a negative impact on plant health or soil quality. The only site where surfactants were observed in depth soil samples was the TX household where graywater was applied for irrigation for more than 30 years. In general, surfactants primarily accumulate in soil surface, and not in deeper soil.

6.2.2. Leaching

In the greenhouse study, less than 19% of surfactants added to columns leached through. However, an increasing trend in surfactants leached through the columns was observed, raising concern over migration of surfactants to groundwater when graywater is applied for irrigation over a long duration. A mass balance on surfactants in the greenhouse study columns showed that 92-96% of added surfactants were biodegraded. While a low percentage of surfactants added to greenhouse columns leached through, a significant increasing trend of surfactants leached from columns was observed over the 17 months duration of the study. More research is required to determine if leaching of surfactants would continue to increase over time.

6.2.3. Sorption and Degradation

Results from field and greenhouse studies showed that most of the surfactants retained in the surface soil. Based on results from the households with new installations, surfactant concentrations, after an initial increase from baseline levels, remained fairly constant over time with some decreases after rainy seasons. Results from greenhouse studies showed that a major portion of parent compounds surfactants (>90%) was degraded. It can be concluded that after application of graywater for irrigation main portion of surfactants will be absorb to the surface soil and then will be degraded more likely due to higher microbial activity in root zone.

In addition, results from this study indicated that in the absence of biodegradation, which usually occurs when soil is saturated and anaerobic, sorption is the main process, which controls the fate and transport of surfactants. Among the surfactants measured in this study, AS and AES had the highest mobility. Results also showed that surfactants with higher EO groups or larger molecular weights have lower mobility and leaching potential. Results confirmed that, since AE has the highest sorption capacity and it is readily biodegradable, it accumulates in surface soil and does not leach to the deeper soil. While, AS and AES-EO1 could access deeper soil and leach faster from the soil columns, AES-EO2 and AES-EO3 required higher volume of water to be transported through soil columns. It was also found that soils with higher OM sorb more surfactants than soils with low OM more likely due to having more available sorption sites due to organic compounds present in the soil.

6.3. Summary of Hypotheses Evaluation

H-1.1. Graywater application causes accumulation of sodium in soil but not to the SAR of 5, the level of concern for plant health, and less than reclaimed wastewater irrigation. Results from field studies (chapters 2 and 3) and greenhouse experiments (chapter 4) confirm this hypothesis. Results showed that sodium accumulated in graywater-irrigated soil samples but SAR was always below 5 in soil samples analyzed in this study. Therefore, graywater irrigation is safe in terms of its effects on soil SAR.

H-1.2. Graywater application has no significant impact on boron concentration in soil to the level concern to plant health. Results from field studies confirm this hypothesis and indicated that graywater application is safe in terms of accumulation of B in soil. However, results from greenhouse experiments revealed that B accumulated in soil and started to leach from the columns after 17 months of continuous graywater application. Of note is graywater application in the greenhouse experiments was continuous which was different from real conditions, where graywater is usually being applied during the irrigation season and soil receives precipitation too.

H-1.3. Graywater irrigation increases nitrogen and phosphorus in the soil. Graywater irrigation did not affect TP in soil samples**.** No significant difference of TP was observed in graywater and freshwater-irrigated soil samples collected from field and greenhouse experiments. However, results from new households confirmed that irrigation with graywater increased levels of TN in soil. In addition, results from greenhouse study raised the concern that continuous application of graywater causes leaching of nitrogen in the form of nitrate from the soil columns.

H-1.4. Graywater application causes accumulation of surfactants and antimicrobials in soil but less than levels found in bio-solid amended soil. Results from both field and greenhouse study confirm that graywater application caused accumulation of surfactants in soil. Concentration of surfactants in soil irrigated with graywater was lower than concentrations reported in sludge amended soils by other literatures.

H-1.5. Similar to reclaimed wastewater application, graywater increases the level of organics in soil after long-term application. Under real condition, no significant difference between OM measured in graywater and freshwater-irrigated soil samples was observed.

H-2.1. After application of graywater for irrigation, surfactants mainly retain in the surface soil and do not leach to deeper soil. Results obtained from field and greenhouse studies confirm that surfactants mainly retained in the surface soil.

H-2.2. Antimicrobials do not leach to deeper soil. Results from the field studies showed that antimicrobials retained in the surface soil more likely due to their strong hydrophobic characteristics. Results indicated that, while leaching of antimicrobials after graywater application is not a concern, accumulation of these compounds at notable concentrations warrant further investigations on their negative effects such as bio-accumulation or causing antibiotic resistant genes.

H-2.3. Continuous irrigation with graywater causes leaching of nitrogen especially in the form of nitrate. At some sampling locations in the field study, higher nitrogen especially in the form of nitrate was observed. Higher growth rate was observed in greenhouse experiments which were more likely attributed to higher available nitrogen in the soil. While adding nitrogen to the soil via graywater application was seemed to be beneficial, no significant difference in leaching of nitrogen was observed from graywater and freshwater-irrigated soil columns in the greenhouse experiments.

H4. Intense surface activity and hydrophobic characteristics render sorption as one of the most relevant processes in surfactants transport through the soil. Results from greenhouse experiments and column studies revealed that adsorption is one of the main mechanisms which control surfactants mobility especially in the absence of biodegradation.

H-2.4. Continuous irrigation with graywater causes leaching of salts. Results from the greenhouse experiments confirmed that leachate collected from graywater irrigated columns had significantly higher TDS than freshwater –irrigated columns. Also, an increasing trend for TDS was observed in the leachate collected from graywater-irrigated columns. Thus, results warrant further investigations for safe application of graywater especially in the locations where groundwater level is higher.

H-3.1. Surfactants within the range of concentrations found in graywater behave similarly in terms of sorption compare to solutions with high concentration of surfactants used in other studies. Results from greenhouse experiments and column studies showed that surfactants behave similarly within the concentrations below 30 mg L^{-1} in terms of sorption compared to higher concentrations used in other studies. However, results showed that surfactant concentration in soil reaches a fairly constant level even after long-term application of graywater for irrigation more likely due to biodegradation of their parent compounds.

H-3.3. There is a correlation between surfactants adsorption and organic content of the soil. Results obtained from columns study confirmed that adding more OM to the soil, increases the retention of surfactants in the soil columns. Also, results from the new households showed a positive correlation between soil OM and surfactants concentration.

H-3.4. There is a correlation between sorption of surfactants and clay content of the soil. While other researchers reported a correlation between clay content of the soil and surfactants concentration, no significant correlation was found in this study. It should be noted that, the ongoing phase of the column study will allow us to draw a more comprehensive conclusion about this correlation.

H-4.1. Graywater has lower surface tension which causes reduction in capillary rise and affects movement of water trough soil pores in unsaturated zone. Theoretically, surfactants reduce the capillary movement of water trough soil in unsaturated zone because due to the reduction they cause in surface tension of water. While we did not conduct a direct experiment to evaluate this hypothesis, results from infiltration tests showed no significant difference between graywater and freshwater-irrigated areas at most of the sampling locations. However, in the greenhouse experiments, soil columns with no plants irrigated with graywater had significantly higher infiltration rate than fresh water irrigated soil columns. More research is required to evaluate the effect of graywater application on soil hydrodynamics. Evaluation of soil hydrodynamics through direct experiments such as soil diffusivity test will provide more data to address this hypothesis.

6.4. Summary of Findings

As a summary on effects of graywater irrigation to soil quality, graywater irrigation resulted in accumulation of surfactants and antimicrobials in soil as well as increased SAR. Surfactant concentration did not increase with duration of graywater irrigation and greenhouse studies showed a large portion of surfactants added are biodegraded. More research is required to determine the impacts of antimicrobial accumulation. While SAR did increase in soil irrigated with graywater, the increase was not high enough in any of the sampling locations to raise concern about soil quality or plant health. Consequently, graywater application seemed to be safe in most of the locations, when soil did not have high levels of SAR and infiltration rate initially.

To summarize the potential for graywater constituents to leach into groundwater, there is a potential for salts, N, B and surfactants to leach through soil when graywater is applied for irrigation. A portion of the applied N is assimilated by plants, but leaching of N was still observed. While a low percentage of surfactants added to greenhouse columns leached through, leaching increased with the duration of the study (17 months). More research is required to determine if leaching of surfactants would continue to increase over time.

6.5. Suggestions for Future Studies

In the course of this study, it has been found that there are some areas require more research.

- Antimicrobials were detected in graywater irrigated areas and not in control areas irrigated by freshwater. Little is known about the impacts of antimicrobials in a soil environment and research is still underway to determine if antimicrobials are linked to formation of antibiotic resistant genes. More research is required to determine the impacts of antimicrobials in graywater irrigated soil.
- Surfactant concentration in leachate samples continually increased over 17 months of application in greenhouse studies. Further work is required to determine if surfactants would continue to leach at a higher rate and if this may pose risk.
- Most of the studies on environmental risk assessment of surfactants have been conducted on water and sediment organisms. Further research is required on risk assessment of surfactants on soil organisms.
- This study was limited in that only 7 households were studied. To rigorously evaluate the fate of graywater constituents under varying conditions, a mathematical model could be developed and run under multiple soil conditions. Such a model may identify some site characteristics not conducive to graywater application. Of the limited sites studied here, conditions were not identified to be unsuitable for graywater application.
- In this research only major graywater contaminants have been studied. Further research is suggested to evaluate fate and transport of other emerging contaminants such as pharmaceuticals which potentially can be found in graywater.

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