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LONG-TERM STUDY ON LANDSCAPE IRRIGATION USING HOUSEHOLD GRAYWATER - EXPERIMENTAL STUDY

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ABSTRACT AND BENEFITS

Abstract:

As water supply becomes more limited throughout the world, there is a growing interest for innovative approaches to water resources sustainability. One approach that is gaining popularity is household graywater reuse for residential landscape irrigation.

Graywater irrigation systems offer many benefits, however the use of such systems has not become widespread due to concerns about safety issues. While some states have begun to legalize and regulate the practice of graywater reuse for residential landscape irrigation, little guidance based on scientific data has been provided for the safe operation of graywater irrigation systems. Limited scientific data is available on the fate of graywater chemical and microbiological constituents and the effect of these constituents on plant health after graywater is applied for irrigation. The objective of this research project was to elucidate information on the fate and occurrence of graywater constituents and their potential impacts on soil quality, groundwater quality, and plant and human health as a result of its application for residential landscape irrigation. This project began in May 2008 and included a series of experimental studies. The experimental studies were conducted in three parts: existing household systems, new household systems, and greenhouse studies. The research team found that most landscape plants are healthy under long-term graywater irrigation compared to freshwater irrigation. Among 22 plant species evaluated, the research team only observed three species (avocado, lemon tree, and Scotch pine) that were sensitive and showed reduced growth, leaf burning, or reduced fruit production under long-term graywater irrigation. In summary, graywater irrigation resulted in accumulation of surfactants and antimicrobials in soil as well as increased sodium. Of note is that the sodium increase after 5 or more years was not high enough in any of the sampling locations to raise concern about soil quality or plant health. There is potential for salts, including nitrogen and boron, to leach through soil when graywater is applied for irrigation.

Benefits:

- Provides science based data on effects of graywater irrigation on soil quality and plant health 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.

Keywords: Graywater irrigation, graywater reuse, leaching, soil quality.

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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
LAS: Linear Alkylbenzene Sulfonates
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

EXECUTIVE SUMMARY

As water supply becomes more limited throughout the world, there is a growing interest for innovative approaches to sustainable water resources. One approach that is gaining popularity is household graywater reuse for residential landscape irrigation. However, there are potential risks associated with this approach, and those risks are largely unquantified. Application of graywater may result in increased levels of pathogens in surface soil, negative impacts to soil quality, potential groundwater contamination, or negative impacts to plant health. Graywater irrigation systems offer many benefits, however the use of such systems has not become widespread due to concerns about safety issues. While some states have begun to regulate the practice of graywater reuse for residential landscape, little guidance based on scientific data has been provided for the safe operation of graywater irrigation systems. Limited scientific data is available on the fate of graywater chemical and microbiological constituents and the effect of these constituents on plant health after graywater is applied for irrigation. The objective of this research project was to elucidate information on the fate and occurrence of graywater constituents and their potential impacts on soil quality, groundwater quality, and plant and human health as a result of its application for residential landscape irrigation.

Experimental studies were conducted in three parts: existing household systems, new household systems, and greenhouse studies. Field studies were conducted on both households with existing systems and households with newly installed systems. Four households were selected in AZ, CA, CO, and TX where graywater was applied for more than five years. In addition, new graywater irrigation systems were installed at three households (AZ, CA, and CO). Baseline samples were collected at the households with newly installed systems prior to initiation of graywater irrigation. Households with newly installed systems were monitored for two to four years. At all households studied, soil samples were collected in areas irrigated with graywater and in a control area with similar vegetation irrigated with a source of freshwater. Plant health was monitored in addition to analysis of tissues to evaluate impacts to plant health. In addition to the field studies, a greenhouse experiment was conducted to evaluate the impact of graywater application to plants and to monitor leachate from graywater irrigated soils.

Results from the field study on existing and new household systems showed that most plants are healthy under long-term graywater irrigation. Among 22 plant species evaluated, the research team only observed three species (avocado, lemon tree, and Scotch pine) that were sensitive and showed reduced growth, or leaf burning, or reduced fruit production under graywater irrigation. Graywater irrigation was found to significantly increase sodium in households with graywater systems in place for more than five years ($P \leq 0.05$), however not to levels of concern for plant health or soil quality. Graywater irrigation was also found to significantly increase surfactants in soil at households with graywater reuse systems in place for more than five years. In addition, soil collected from households with newly installed graywater systems had significantly higher surfactant concentration than control areas irrigated with graywater ($P \leq 0.05$). Surfactant concentration did not continually increase with duration of graywater irrigation. The antimicrobials triclosan and triclocarban were detected in graywater irrigated areas, but not freshwater irrigated areas. These constituents were only detected in

surface soil samples and are not easily transported through soil. Graywater has the potential to contaminate the environment with human-associated fecal organisms, including *E. coli* and enterococci. In this study, however, the research team found no strong, consistent effect of graywater on numbers of *E. coli* or enterococci in soil. Contamination was inconsistent and depended on the household, sampling date, and depth of soil sampled. In addition, *E. coli* and enterococci were detected in freshwater-irrigated soils, indicating sources other than graywater for fecal indicators detected in the environment. Of note is that these organisms can grow in the environment.

The objective of the greenhouse study was to evaluate the potential for graywater constituents to leach through soil and contaminate groundwater. There is a potential for salts, and in particular nitrogen (N) and boron (B) salts to leach through soil when graywater is applied for irrigation. A portion of the applied N is taken up by plants, but leaching of N was still observed. Leaching of N was lower in columns planted with grass compared to shrubs. Of note is that nitrate measured in graywater is well below the National Primary Drinking Water Standard limit of 10 mg L^{-1}. While a low percentage of surfactants added to greenhouse columns leached through 50 cm, leaching did increase with the duration of the study (17 months). More research is required to determine if leaching of surfactants would continue to increase over time. More than 90% of applied surfactants were determined to be biodegraded in planted columns. Due to the much greater nutrient content in the synthetic graywater for the greenhouse study, synthetic graywater-irrigated plants exhibited greater plant biomass and enhanced density, color, and quality when compared to potable water irrigated plants. No visual symptoms of toxic effects were observed in the greenhouse study. It is thus implied that surfactant accumulation in planted column soil did not result in phytoxicity. Graywater irrigation resulted in higher infiltration rates in columns compared to potable water irrigated columns.

No major concerns were identified in this study that would render reuse of graywater following best management practices unsafe for growing garden plants. Considering human health, the state of Arizona has set the standard for graywater irrigation best management practices (http://www.azdeq.gov/environ/water/permits/download/graybro.pdf) and these practices are recommended in many states. Graywater does contain pathogens and human contact with graywater should be avoided. Graywater should be applied through drip irrigation with a protective layer of mulch above emitters. In some states, subsurface irrigation systems are required. One such system was studied as part of this research. There was no indication that a subsurface irrigation system resulted in lower indicator organisms compared to surface irrigation systems studied here. In general, the source of indicator organisms was difficult to determine since they were found in areas irrigated with freshwater (control). However, because indicator organisms were detected in graywater irrigated areas, it is recommended that human contact with graywater irrigated areas be avoided. Placing a mulch layer over drip emitters where graywater is applied appears to be a good control to minimize human contact with graywater irrigated soil. The research team found that most plants were healthy under long-term (more than 5 years) graywater irrigation. However, avocado, lemon tree, and Scotch pine are sensitive to graywater irrigation and not recommended when graywater is the only source of irrigation water. Results from the greenhouse study showed that N present in graywater was beneficial for plant growth. Supplemental fertilizer can be reduced or eliminated where graywater is applied for irrigation.

CHAPTER 1.0
INTRODUCTION

As communities throughout the United States and abroad are becoming interested in innovative approaches to sustainable water resources, household graywater reuse for residential landscape irrigation is gaining popularity. In a typical household, graywater (near 28 gallons per person per day) is nearly 50% of the total wastewater generated. 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. 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%). Some states, including California, Arizona, and New Mexico have regulated the practice.

There are potential risks associated with graywater reuse for irrigation. The physical, chemical, and microbial characteristics of graywater are highly variable based upon the sources connected to the collection system, household inhabitants, household chemicals used by the residents for personal hygiene and house cleaning, personal care, plus medications and waste products disposed of in sinks (Eriksson et. al., 2002). Application of graywater may result in negative impacts to plant health, negative impacts to soil quality, increased levels of pathogens with human health implications, or potential groundwater contamination with chemical and viral constituents present in graywater. The potential risks were evaluated and methods of graywater application that minimize these risks were explored.

1.1 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 a range of plant species.

1.2 Graywater Impacts to Soil Quality and Groundwater

In addition, application of graywater for irrigation may impact soil chemistry. When graywater is reused for irrigation, chemical constituents of concern include nutrients (nitrogen (N) and phosphorus (P)), metals, total salts, boron (B), and personal care product ingredients. 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. 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 does contain elevated sodium compared to potable water (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. B 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, limited research to date has shown negative effects of graywater irrigation from the point of B accumulation in soil (Gross et al., 2005).

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. In addition, surfactants applied in graywater may be transported to groundwater. 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. An emerging 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.

Graywater constituents may impact groundwater quality in addition to soil quality if the constituents are transported to groundwater. Nutrients and organics (surfactants, antimicrobials, etc.) are of particular concern for environmental quality and human health. While data is available on leaching of chemical constituents in reclaimed wastewater through soil, such data is not available for graywater irrigation. 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.

1.3 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. Pathogens include disease-causing viruses, bacteria, protozoa and helmiths. 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.

1.4 Project Objective and Approach

While graywater reuse for household irrigation is widespread, potential effects on soil quality, groundwater quality, and plant health 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. 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 present in graywater may persist and pose human health risks. Current research has not 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 study proposed herein describes scientific experiments to alleviate these information gaps regarding household graywater irrigation.

Phase 1 of the project, a literature review and synthesis, was completed in March 2006 and is available from Water and Environment Research Foundation (WERF; Roesner et al., 2006). The final report contains a comprehensive synthesis of the current state of the knowledge on graywater reuse for landscape irrigation at the household level. The report also identifies information gaps for future research, a number of which are being addressed through Phase 2. The objective of this project (Phase 2) was to elucidate information on the fate and occurrence of graywater chemical constituents and pathogens and their potential impacts on soil quality, groundwater quality, and plant and human health as a result of its application for residential landscape irrigation. Field studies (Chapter 2) were the focus of research efforts to ensure data was collected that can be directly used by regulatory agencies and home owners interested in graywater irrigation application. Because field conditions are highly variable rendering data interpretation complex, a set of greenhouse studies was conducted to determine the fate of graywater constituents when applied for irrigation (Chapter 3). Leachate water quality and soil quality were both evaluated during the greenhouse experiments. Quantitative data collected on the fate of graywater constituents and effects on plant health will provide scientific data that has been lacking on the impacts of graywater reuse. This information should be of use to those who make decisions on graywater regulations.

CHAPTER 2.0

FIELD EXPERIMENTS ON LONG-TERM EFFECTS OF GRAYWATER REUSE

2.1 Introduction

The objective of field experiments was to elucidate information on the fate and occurrence of graywater chemical and microbial constituents and their potential impacts on soil quality, groundwater quality, and human and plant health. Field experiments included two parts. First, soil and plant samples were collected from several household sites that have been using graywater for irrigation for more than 5 years and compared with analogous soil and landscaping that has been irrigated with potable water. Since it was expected that the operating protocols for these systems were not well documented, the second part of the study included new applications of graywater to selected sites. These sites were operated in a controlled manner for 1-2 years to determine changes to soil and plant health that might occur due to graywater irrigation. During these field experiments, graywater samples, soil samples, and plant samples were collected at each sampling location.

2.2 Experiment Setup

A total of seven households were included in this study. Four households with existing graywater system were included in the first part of the study, located in Bisbee, AZ, Escondido, CA, Fort Collins, CO, and Dallas, TX (Table 2-1). One sampling event was conducted at both the CA and AZ sites, two sampling events were conducted in TX, and three in CO. Sampling events occurred near the end of the dry season in each location, when accumulation of graywater constituents in soil would have been highest. Three households, with newly installed graywater systems included in the second part of the study, were located in Phoenix, AZ, Cotati, CA, and Fort Collins, CO. Six sampling events were conducted in AZ, four sampling events were conducted in CA and five sampling events were conducted in CO, respectively. At the households with existing graywater systems, only soil samples were collected. At the households with newly installed graywater system both soil and graywater samples were collected. 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. Plant samples were collected from both graywater and freshwater irrigated areas at each household. Of note is that irrigation in the control areas varied (Tables 2-2 and 2-3). Control area irrigation water is referred to as freshwater throughout Chapter 2 and of note is that freshwater was not necessarily potable water.

Table 2-1. Summary of Sampling Events.

Location	Sampling Event	Date
Existing Systems		
Bisbee, AZ	Year 2	6/30/2009
Escondido, CA	Year 1	10/20/2008
Fort Collins, CO	Year 2	10/6/2009
	Year 3	9/22/2010
	Year 4	10/3/2011
Dallas, TX	Year 1	9/15/2008
	Year 2	10/20/2009
New Installations		
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

2.2.1 Description of Households with Existing Systems

A summary of the graywater systems at households studied where graywater was applied for irrigation for five years or more is included in Table 2-2.

Table 2-2. Summary of Graywater Systems at Households with Existing Systems.

Location	Duration of Graywater Irrigation (years)	System Description	Irrigation Method	Irrigation Frequency	Source of Irrigation Water in Control Area
Escondido, CA	10	Storage, slow sand filter, pump	Submerged Drip	Daily	Municipal
Fort Collins, CO	5	Storage, course filter, pump	Hose Application	Manual application as needed	Municipal
Dallas, TX	31	No storage, direct connect from washing machine	Hose Application	With operation of washing machine	Municipal
Bisbee, AZ	5	No storage	Collected and Applied in Buckets	Manual application as needed	Harvested Rainwater

2.2.1.1 Arizona

The household sampled with an existing system in Arizona is located in Bisbee. At this household, graywater runs through pipes outdoors and was collected in buckets (Figure 2-1). The buckets were then carried throughout the yard and water manually applied. The homeowner was extremely careful not to overwater and regularly tested soil moisture prior to graywater application. . Depth sampling was not possible at this site and water was visually observed to spread laterally after application to the soil, almost like pouring water on concrete. Therefore, soil samples were collected around the perimeter of a salt bush that had been irrigated with graywater for six years. Samples were collected within a 0 - 2' , 2' - 3', and 3' -4' radius of the perimeter. Another sample was collected from a nearby salt bush that had only been irrigated for one year with graywater but had received more graywater in the last year than the other sampled salt bush. The homeowner claimed that the entire yard was covered with trash and served as a junk yard prior to construction of their home 8 years ago.

Figure 2-1. Graywater Collection System at Bisbee, AZ (existing system).

2.2.1.2 California

The household with an exisiting graywater irrigation system in California is equipped with a more advanced irrigation system than other households studied here (Figure 2-2). The system was comprised of a slow sand filter which is automatically backwashed daily, pump, and submerged drip system. Underground emitters were placed in the root zone of graywater irrigated plants. The installed emitters were very effective in that they prevent clogging resulting from root growth. The system was installed by ReWater Systems, Inc. No operational problems have been encountered with this system in its 10 years of operation. Landscape was irrigated on a daily basis. Depth sampling was not feasible due to a layer of bedrock present at around 4-8" below ground surface. The highly sloped terrain at this sampling location rendered soil quality data difficult to interpret, due to the potential for graywater constituents to migrate to freshwater-irrigated areas.

Figure 2-2. Graywater Reuse System (Rewater®) in Escondido, CA(existing system).

2.2.1.3 Colorado

The household with an existing system in Colorado was equipped with a simple irrigation system consisting of a storage tank, pump, and coarse filter (Figure 2-3). Depth sampling was feasible at this household. Of note is that while this homeowner does not have pets, the yard

Figure 2-3. Graywater Storage Tank at Existing Household in Fort Collins, CO (existing system; Photo by J. Bergdolt)

backs to open space and there is a presence of wildlife, particularly birds and geese in the area.

2.2.1.4 Texas

Samples were collected from a household in Texas where wastewater from a laundry machine has been drained to landscape for more than 30 years. Turf grass in the graywater irrigated area appeared very healthy (Figure 2-4). Of particular note is that free range chickens and a dog defecate in the area irrigated with graywater.

Figure 2-4. Graywater Application from Hose Directly Connected to Washing Machine in Dallas, TX (existing system).

2.2.2 Description of Households with Newly Installed Systems

A summary of the graywater systems at households studied where graywater systems were newly installed is included in Table 2-3.

Table 2-3. Summary of Graywater Systems at Households with Newly Installed Systems.

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

2.2.2.1 Arizona

At the Arizona household where a graywater irrigation system was installed, graywater was collected in the home and pumped to the roof top where it then gravity flowed down to the yard (Figure 2-5). No storage was in place. Of note was that the control area was irrigated with flood water from a nearby irrigation canal. Soil samples were collected from a graywater-irrigated area within a small (5-10 ft^2) patch of turfgrass installed by the homeowner specifically for this study. The turfgrass was brought onto the site as sod, it was not planted within the existing native soils. This means that two distinctly different soils may be present within the graywater soil samples, one type that is the soil in which the turfgrass was planted and grown and another type that is the native soils below. The homowners allowed a dog into both graywater and freshwater-irrigated areas and freerange chickens were present mostly in the backyard where graywater irrigation took place.

Figure 2-5. Graywater Pipes to Gutter for Irrigation in Phoenix, AZ (new installation).

2.2.2.2 California

A graywater system was installed at a household in California where graywater was discharged to soil through an infiltrator (Figure 2-6). Manure amendments were applied to soil in landscaped areas near to graywater infiltrators, rendering interpretation of plant health difficult. However, the grass area where soil samples were collected was not impacted by the amendment and soil data is included for interpretation in this report.

Figure 2-6. Subsurface Infiltration of Graywater in Cotati, CA (new installation).

2.2.2.3 Colorado

The system currently consists of a 55-gallon barrels that collect graywater from a washing machine, showers and bathroom sinks (Figure 2-7). The graywater is gravity-distributed to an irrigated area using perforated hoses. The homeowners do have a dog that accesses the backyard and some dog defecate was present near the sampling locations, potentially impacting presence of indicator organisms. Some plants were damaged by the dog in the backyard. In addition, the backyard where graywater applied was particularly shadier than the front yard, where freshwater was applied for irrigation. Therefore, interpretation of plant health at this site is omitted from this report. However, these factors did not impact interpretation of soil quality data and data collected on soil quality is presented in this report.

Figure 2-7. Outdoor Storage Tank with Gravity Feed of Graywater Through Hose in Fort Collins, CO (new installation).

2.2.1 Sample Collection and Analysis

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. Samples were collected as close as possible to the base of a plant that was irrigated with either graywater or freshwater. 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 households in CA (existing and new households) or AZ (existing household) due to a shallow layer of soil above bedrock or 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 collected at the households with newly installed graywater systems. Graywater samples were analyzed for general water quality parameters, indicator organisms, surfactants and antimicrobials. Methods for analysis are outlined in the Quality Assurance Project Plan (QAPP) delivered to WERF at the start of the project (Appendix A).

Plant evaluation was also conducted at each location to asses differences between the graywater irrigated and freshwater irrigated landscape plants. The analysis included plant identification, evaluation, and sample collection for tissue analysis. In addition, landscape plants were evaluated for their health. Plant types examined in this study included typical trees, shrubs, bedding plants, and turfgrasses.

For data collected from the existing households, significance of the effect of graywater irrigation on soil surfactant concentration, SAR, and *E. coli* was determined at the 95% confidence interval using a 2-way analysis of variance (ANOVA). Replicate samples were not

collected at any sampling event and were collected more than one time only at the CO and TX households (Table 2-1). The 2-way ANOVA was applied with irrigation type and sampling event as the two factors to address variability among sampling locations and at the same location, but different time. Of note is data collected from CA sampling location with existing graywater system was not included in the 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. At households with new graywater irrigation systems installed, multiple samples were collected at the same household. Therefore, 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; $P \leq 0.05$).

2.3 Effects on Plant Health

2.3.1 Households with Existing Systems

The research team evaluated and collected plant samples from four households in different locations (CO, TX, CA, and AZ) that have been using graywater for irrigation for many years and compared those plant samples with those that have been irrigated with fresh water. The households in CA, TX, and AZ were evaluated once in 2008 – 2009. The household in CO was evaluated twice (first in 2008 and again in 2010). Plants were evaluated for the following criteria: crown density, dieback, foliage color, foliar burn, foliar necrosis, leaf size, insect and disease presence, and overall quality. For evergreen conifers, the research team also collected data on the number of years of needle retention and year-to-year growth increments. Based upon the overall evaluation, plants were classified for their relative tolerance levels to the use of graywater irrigation. Plants that exhibited some improvements or no changes under graywater irrigation were placed in tolerant category. Plants that appeared healthy with only slight change in 1-2 evaluation criteria were placed in moderate tolerant category. Plants that exhibited small degree of decline were placed in moderate sensitive category whereas plants that exhibited significant decline were ranked as sensitive to graywater irrigation.

The researcher's evaluations demonstrated that while most of the plants evaluated for this study were tolerant to graywater irrigation, some were found to be sensitive (Table 2-4). Tolerant plants were healthy and did not exhibit any apparent changes with regards to growth, leaf size, color, canopy density, number of blooms when graywater was used as the irrigation source.

Table 2-4. Relative Tolerance of Landscape Plants to Graywater Irrigation in the Existing Household Study.

Tolerant (Figure 2-8)	**Moderately Tolerant**	**Moderately Sensitive**	**Sensitive** (Figure 2-9)
Hackberry (*Celtis occidentalis)*	California Valeriana (*Valeriana californica*)	Himalayan border jewel (*Polygonum affine*)	Scotch pine (*Pinus sylvestris*)
Four-wing saltbush (*Atriplex canescens*)	Plum tree (*Prunus spp*.)	Mugo pine (*Pinus mugho*)	Hass avocado (*Persea americana* 'Hass')
Globe mallow (*Sphaeralcea ambigua*)		Bearded iris (*Iris germanica*).	Lemon tree (*Citrus limonium*).
Honey mesquite (*Prosopis glandulosa*)			
Desert daisy (*Bahia absinthifolia*)			
Juniper (*Juniperus spp*), Euonymus (*Euonymus spp*.)			
Rose of Sharon (*Hisbiscus syriacus*)			
Chrysanthemum (*Chrysanthemum spp.)*			
St. Augustine grass (*Stenotaphrum secundatum*)			

Figure 2-8. St. Augustine Grass (Upper Panel), Rose of Sharon (Middle Panel), and Euonymus under Freshwater Irrigation (Left Panel) and Graywater Irrigation (Right Panel). These Plants Exhibited Some Improvements or No Changes under Graywater Irrigation.

Figure 2-9. Hass Avocado Under Freshwater Irrigation (left panel) and Graywater Irrigation (Right Panel). Graywater Irrigation Had Reduced Leaf Size, More Severe Wilting and Much Reduced Fruiting.

Leaf samples for all the plants described above were collected. The dominant influence of mineral content in leaves was plant species, i.e. different species differed dramatically in plant tissue mineral content (Appendix B, Table B-1). A few consistent trends were found regarding the influence of irrigation water source on individual leaf mineral content. For the AZ site, graywater irrigated plants had a lower K/Na ratio than the controls. For both graywater irrigation and control, Saltbush and Desert Daisy exhibited the highest K/Na ratio of all species. Tree Tobacco and Honey mesquite had relatively low K/Na ratio under graywater irrigation than other species. Since higher tissue K/Na ratio often indicates less sodium and/or salinity stress, Saltbush and Desert Daisy had more favorable K/Na ratio than Tree Tobacco and Honey mesquite. At the CO site, the research team found that graywater-irrigated Euonymus, Rose of Sharon, and Himalayan Border Jewel exhibited higher Cl and Na content than freshwater-irrigated plants for samples collected in 2010.

2.3.2 Households with New Installations

From the household in Phoenix, AZ, the research team evaluated and collected plant samples four times: October 2008, June 2009, January 2010, and June, 2010. This household is located in a 1950s suburban neighborhood and the landscape was segmented into graywater and freshwater irrigation sections for comparison. In June and October, most plants were green. In January, most cool season plants exhibited new growth. Warm season plants (such as bermudagrass and lemongrass) had signs of dormancy.

Plants were evaluated for crown density, dieback, foliage color, foliar burn, foliar necrosis, leaf size, insect and disease presence, and overall quality. Graywater irrigation had positive impacts on bermudagrass (*Cynodon dactylon* L.), Peach (*Prunus persica*), and Black-eyed Susan (*Rudbeckia hirta*). Under graywater irrigation, these plants consistently exhibited higher shoot growth, better density, color, less degree of winter dormancy and overall quality compared to those irrigated with freshwater. Canna lily (mixed varieties) (*Canna spp*.) did not show differences between graywater and control treatments. Graywater irrigation had negative impacts on lemon (*Citrus limonium*) and hybrid Rose (*Rosa spp*.). After two years of graywater

irrigation, lemon trees exhibited less stem elongation and more foliar burn at the leaf margins. Graywater-irrigated hybrid rose showed 10-20% more powdery mildew.

Despite the fact that two years of irrigation with graywater is a short period for a confident assessment of tolerance level, our field evaluation suggested that bermudagrass, peach, Black-eyed Susan, and Canna lily were tolerant to graywater irrigation. Lemon and hybrid rose were sensitive to graywater irrigation. These results are in agreement with findings from the existing household study (Section 2.4.1).

In 2008, 2009, and 2010, plant samples were analyzed for the mineral content. Leaf samples for all the plants described above were collected. Samples were processed and analyzed using methods described in the existing households plant tissue analysis (Appendix B, Table B-2).

Graywater-irrigated bermudagrass, lemon tree, peach tree, lemongrass, and canna lily all had higher chloride content than the control samples collected in January 2010 (Appendix B, Table B-2). Graywater-irrigated bermudagrass, lemon, and peach tree leaves also had higher sodium content (Appendix B, Table B-2). Interestingly, the research team did not see persistent trends for samples collected in June 2009 and 2010.

From the household in Cotati, CA, the research team evaluated and collected plant samples three times: baseline and 1 and 2 years after graywater application. However, the graywater plants were heavily mulched with horse manure and straw at the bases of the plants by the home owner. For the Fort Collins, CO household, the research team evaluated and collected plant samples twice (September 2010 and 2011). At this location the graywater-irrigated plants are located along a border fence under a canopy of several established trees in the back yard. Plants in the front yard of the house are subjected to freshwater irrigation. The confounding environmental factors in CA and CO prototype households made the comparison of plants irrigated with freshwater and graywater inconclusive. The results of plant tissue analyses were summarized in Appendices B-3 and B-4, respectively.

2.3.3 Summary

In this project, the approach of using landscapes that have been using graywater for irrigation for many years were effective in evaluating plant health and response to graywater irrigation. The research team found that most plants were healthy under long-term graywater irrigation. Among 22 plant species evaluated, the research team only observed three species (Avocado, Lemon tree, and Scotch pine) that were sensitive and showed reduced growth, or leaf burning, or reduced fruit production under graywater irrigation. The research team did not observe consistent Na, Cl, and B accumulation in most of the field evaluated species.

For the new prototype household study, the AZ site provided the most reliable results. Graywater irrigation had positive impacts (higher shoot growth, better density, color, less degree of winter dormancy and overall quality) on bermudagrass, peach, and black-eyed Susan. Canna lily did not show differences between graywater and control treatments. Graywater irrigation had negative impacts on lemon and hybrid Rose. For the CA and CO new household sites, confounding environmental factors (such as manure application to graywater irrigated plants and different light exposure) made the comparison of plants irrigated with freshwater and graywater inconclusive.

2.4 Graywater Quality

Graywater samples were collected from households with newly installed graywater systems and analyzed for general water quality parameters (Table 2-5) in addition to surfactants and antimicrobials (Table 2-5). AZ graywater includes 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 2-5).

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 2-6). Trace concentrations of antimicrobials including TCS and TCC were also found in graywater (Table 2-6).

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 2-7; 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 2-7; 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 new graywater irrigation systems (Table 2-5; Ayers and Westcot 1994). In addition these graywater sources had B levels below 0.7 mg L^{-1}, the level which causes toxicity problem in the soil (Ayers and Westcot 1994). The only sample collected from AZ sampling location had TN of 73.8 mg L^{-1}, high above the restricted level of use as 30 mg L^{-1}. This result may be caused by existence of kitchen water in the graywater at this household.

Table 2-5. Quality of Graywater Samples. (n: number of sampling events)

Parameters	AZ (n=1)	CA (n=3)	CO (n=3)
pH	6.6	7.5±0.6	6.7±0.4
	---------- $\mu S\ cm^{-1}$ ----------		
EC	1654	1212±748	945±85
	---------- Eh, mV ----------		
ORP	220	162±20	159±14
	---------- $mg\ L^{-1}$ ----------		
BOD_5	317	214±13	178±25
COD	580	391±13	349±39
DOC	271	220±10	173±14
TSS	75	27±8.5	35±4.7
TDS	930	571±38	354±92
TN	73.8	27.3±2.8	23.0±1.2
NH_4-N	64.9	18.6±1.6	15.4±2.0
NO_3-N	1.35	0.9±0.1	0.5±0.1
Total P	16.4	7.0±2.3	6.0±1.6
PO_4-P	18.2	8.8±2.0	8.7±3.8
SO_4	282.0	100.8±14.7	59.0±15.4
Cl	26	33.5±4.9	21.7±4.0
Br	0.9	0.6±0.1	0.5±0.2
Ca	8.9	9.1±1.6	5.3±0.7
Mg	4.9	3.7±0.8	3.4±0.4
Na	35	39.0±11.3	40.2±4.1
K	9.7	9.5±2.5	13.7±4.2
Co	≤0.01	≤0.01	≤0.01
Cu	≤0.01	≤0.01	≤0.01
Fe	0.01	≤0.01	≤0.01
Zn	0.15	≤0.01	0.15±0.04
Ni	≤0.01	≤0.01	≤0.01
V	0.01	≤0.01	≤0.01
Mo	≤0.01	≤0.01	≤0.01
Cr	≤0.01	≤0.01	≤0.01
Se	≤0.001	≤0.001	≤0.001
B	0.04	0.05±0.02	0.07±0.03
SAR	2.3	2.8±0.5	3.3±0.8

Table 2-6. 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

Table 2-7. 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

2.5 Effects on Soil Quality

General soil quality parameters for all sampling locations are located in Appendix C, and interesting results are highlighted here.

2.5.1 Accumulation of Sodium (Na) and Boron (B)

2.5.1.1 Households with Existing Systems

SAR is a measure of Na concentration relative to other salts (Ca and Mg), and thus provides an index of sodic conditions in soil. Sodium accumulation has been a problem for reclaimed water irrigation (Qian and Mecham 2005) and is also a potential concern for graywater irrigation. Graywater SAR is expected to range from 2.3-5.9 based on values from the literature and graywater samples analyzed in this study (Table 2-7). SAR and EC varied among sampling locations with existing graywater systems and irrigation water treatment (Figure 2-10). SAR was not notably different at the CA and CO sampling locations in the graywater-irrigated area compared to the freshwater-irrigated area. In AZ, Na concentrations were below the limits of detection, resulting in SAR near zero. 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. In contrast, 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 (Figure 2-10).

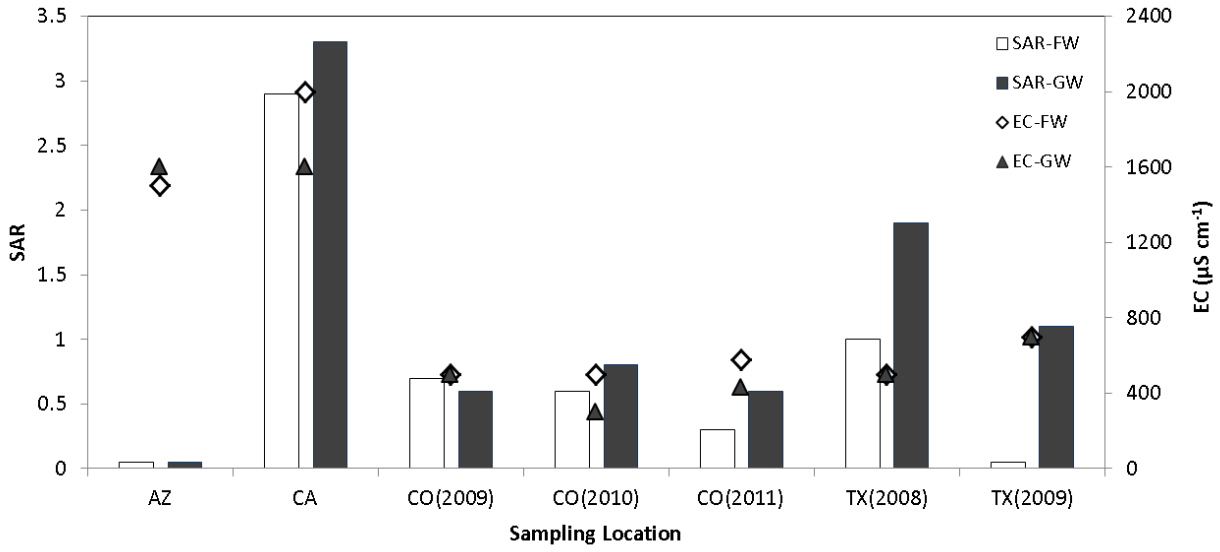

Figure 2-10. SAR and EC in Soil Samples Collected from Households with Existing Graywater Systems.

The household with an existing system in TX had the longest history of graywater irrigation and provides one case study where there were significant differences in several soil properties (soil organic matter content, SAR and B concentration) between graywater- and freshwater-irrigated soils ($P \leq 0.05$). At the TX household with an existing system, the SAR ratio in surface soils (0-15 cm) was greater under graywater irrigation, with values 2-to-22 times those measured in control soil (Figure 2-8).

Hot water extracted B varied among sampling locations, and even between years at the TX and CO households (Figure 2-11). Hot water B concentrations in TX were 45-50% greater in graywater-irrigated soil than in freshwater-irrigated soil despite the large inter-annual variability. Inter-annual variability may be a result of differing soil conditions among sample dates which can impact extraction of B from soil. Otherwise, hot water extractable B concentrations were similar between the two soil areas (CA and CO 2009; Figure 2-11), or slightly lower in the graywater-irrigated soil (AZ and CO 2010; Figure 2-11). In 2009, hot water-extractable B concentrations in graywater-irrigated soil collected from TX exceeded levels measured in freshwater-irrigated soil, down to 90 cm in depth. 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). Plant species sensitive to B can certainly show toxicity symptoms at lesser concentrations, including those measured at this household. At the 2009 sampling event at TX, graywater-irrigated soil samples collected from 0-15, 15-30, 30-46 and 46-61 cm had hot water extractable B concentrations higher than 5 mg kg^{-1} (Figure 2-11). Of note is soil samples collected from 0-15 cm freshwater-irrigated area in TX (2009) and CO (2010) also contained hot water extrable B above 5 mg kg^{-1} (Figure 2-11).

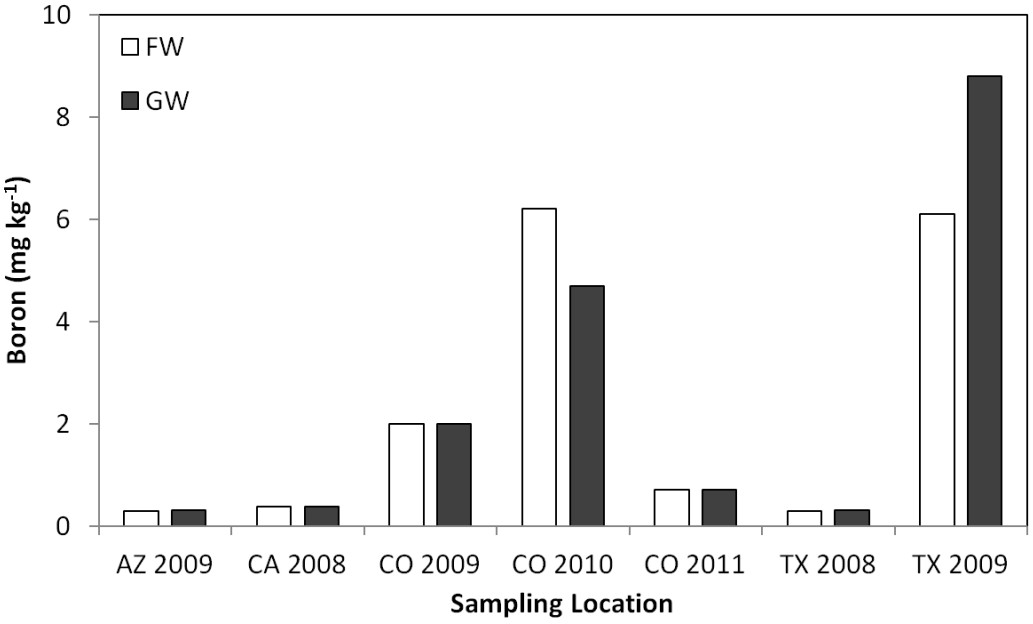

Figure 2-11. B Measured in Surface Soil Samples Collected from Households with Existing Graywater Systems.

2.5.1.2 Households with Newly Installed Systems

Water quality data (SAR and EC; Table 2-5) indicates that graywater irrigation water collected from households with newly installed systems SAR ranged from 2.3-5.9 while EC varied from 900-1700 $\mu S\ cm^{-1}$, and thus would be categorized as none to slight or moderate restrictions for use as irrigation water based on Ayers and Wescott (1994). Among the sampling locations with new graywater systems, highest SAR was measured at the AZ sampling location (Figure 2-12). 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 different was observed for SAR in the graywater-irrigated areas compared to the freshwater-irrigated areas at these households ($P>0.05$; Figures 2-13 and 2-14). 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 (Figures 2-12 through 2-14) 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.

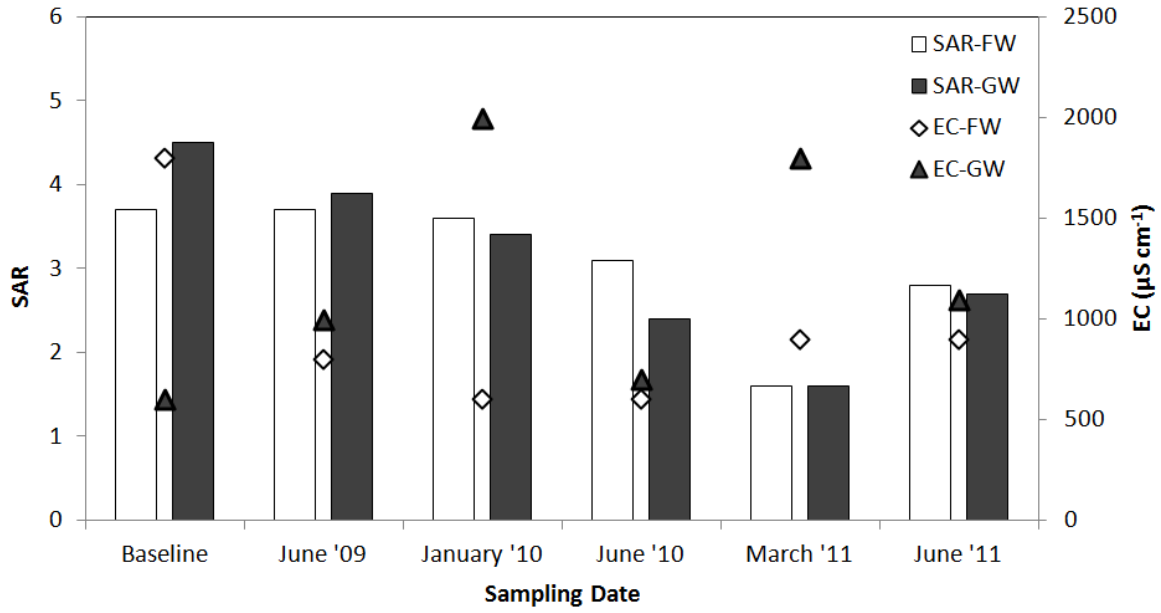

Figure 2-12. SAR and EC Measured in Surface Soil Samples Collected from AZ Sampling Location with New Graywater System. (FW: freshwater-irrigated, GW: graywater-irrigated)

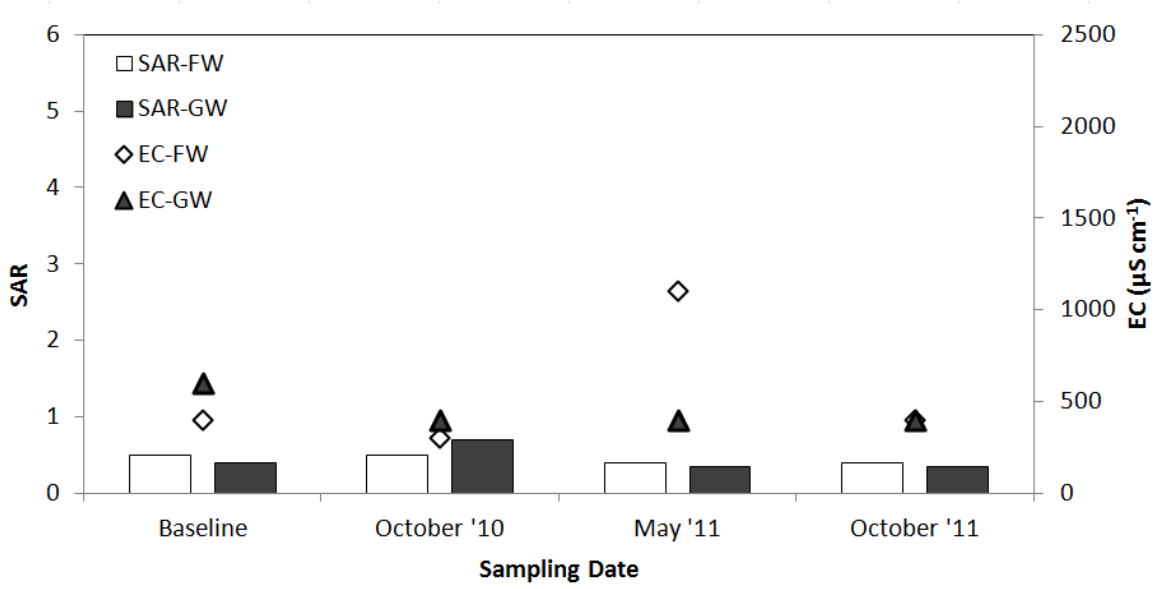

Figure 2-13. SAR and EC Measured in Soil Samples Collected from CA Sampling Location with New Graywater System. (FW: freshwater-irrigated, GW: graywater-irrigated)

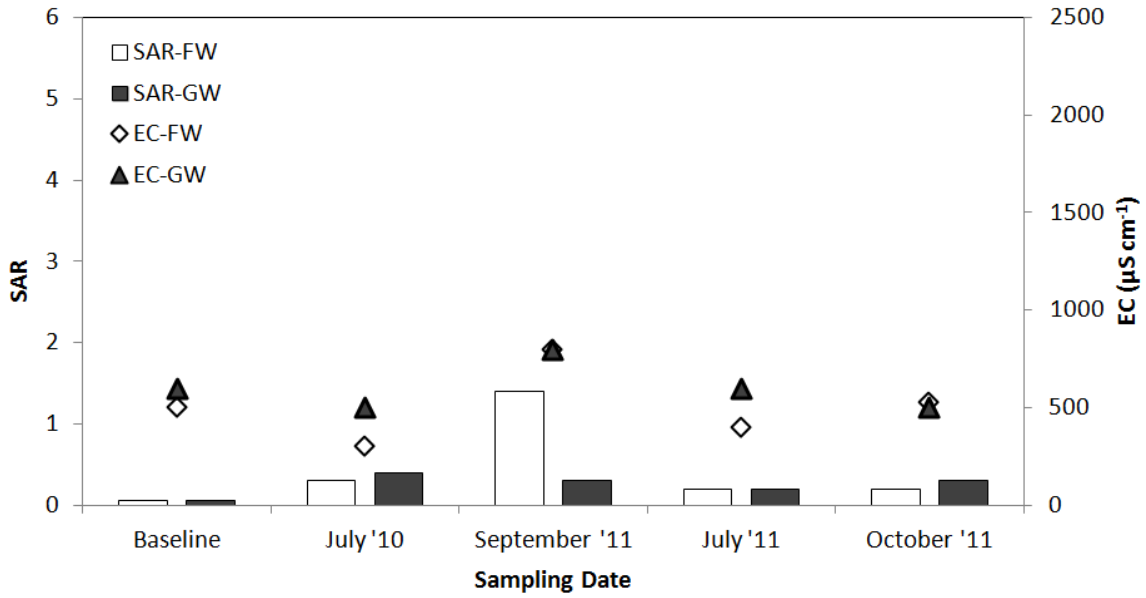

Figure 2-14. SAR and EC Measured in Soil Samples Collected from CO Sampling Location with New Graywater System (FW: freshwater-irrigated, GW: graywater-irrigated)

Hot water extractable B varied among sampling locations, and even between years at the AZ, CA and CO households with newly installed graywater systems (Figure 2-15). However, no accumulation of B was observed in the areas irrigated with graywater during the course of this field study. Except graywater and freshwater-irrigated soil samples collected from AZ in June 2010 and freshwater-irrigated soil sample collected from CO in July 2010, hot water extractbable B was always below 5 mg kg^{-1}.

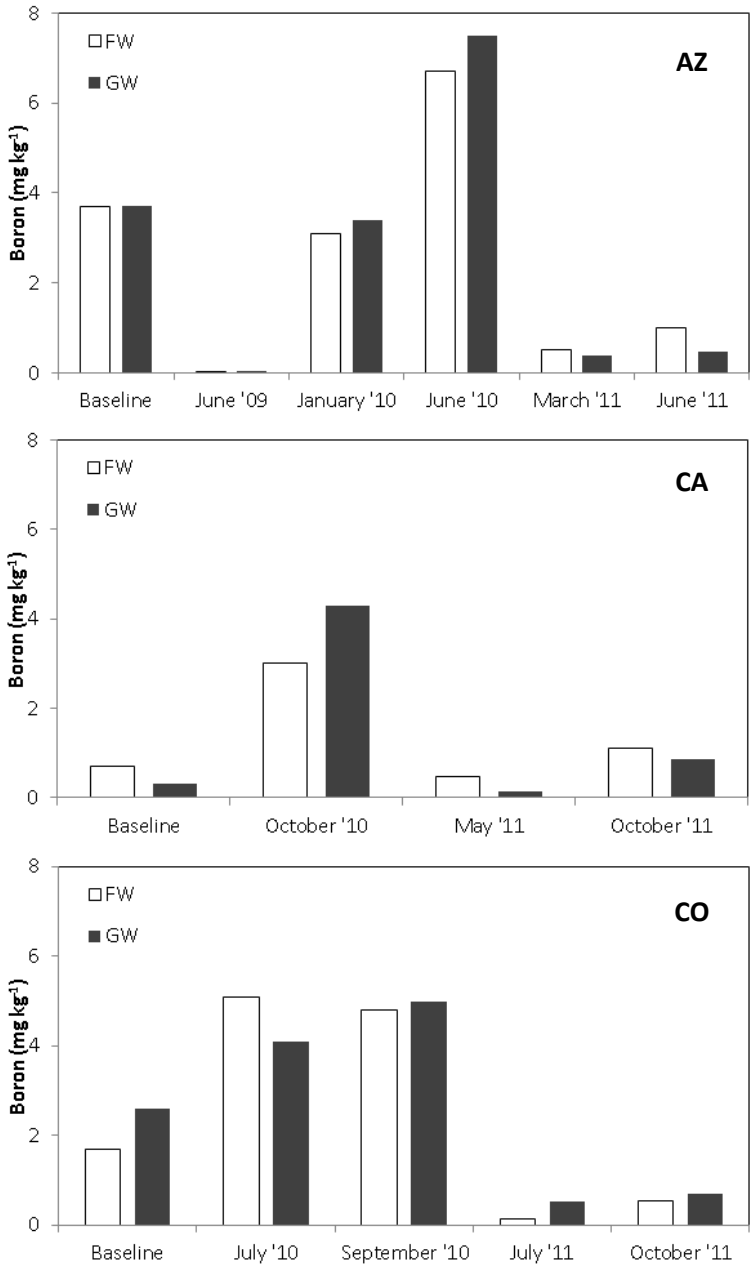

Figure 2-15. B Measured in Surface Soil Samples (0-15 cm) Collected from Sampling Locations with New Graywater Systems

2.5.1.3 Summary

Results from the existing households indicated a significant impact of graywater irrigation on increased SAR compared to freshwater irrigated areas ($P \leq 0.05$). For the households with newly installed systems, 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.

2.5.2 Impact to Organic Content and Nutrients

2.5.2.1 Households with Existing Systems

OM in the surface soil (0-15 cm) was greater under graywater irrigation compared to freshwater irrigation at the TX household (Appendix C, Table C-4). OM varied in soil samples collected from AZ, CA and CO sampling locations with existing graywater systems. While at some sampling events graywater-irrigated areas had higher OM in other sampling events freshwater-irrigated areas had higher OM (Appendix C, Tables C-1 to C-3). In 2008, OM content was 7.3% in graywater-irrigated soil compared to 2.8% in freshwater-irrigated soil collected from the TX sampling site. In 2009, the values were 4.5% in graywater-irrigated and 2.5% in freshwater-irrigated soil. TN and TP varied among graywater and freshwater irrigated sampling locations and no consistent trend was observed for nutrients (Appendix C; Tables C-1 to C-4).

2.5.2.2 Households with Newly Installed Systems

In AZ and CO, graywater-irrigated surface soil contained 20-50% and 35-53% more OM compared to soil receiving freshwater respectively (Appendix C, Tables C-5 and C-7). OM levels were not notably different at the CA sampling location in the graywater-irrigated area compared to the freshwater-irrigated area.

The AZ and CO households with new graywater systems were the only households where soil NO_3-N levels were elevated under graywater irrigation over time (Figures 2-16 and 2-17). 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 ($P \leq 0.05$).

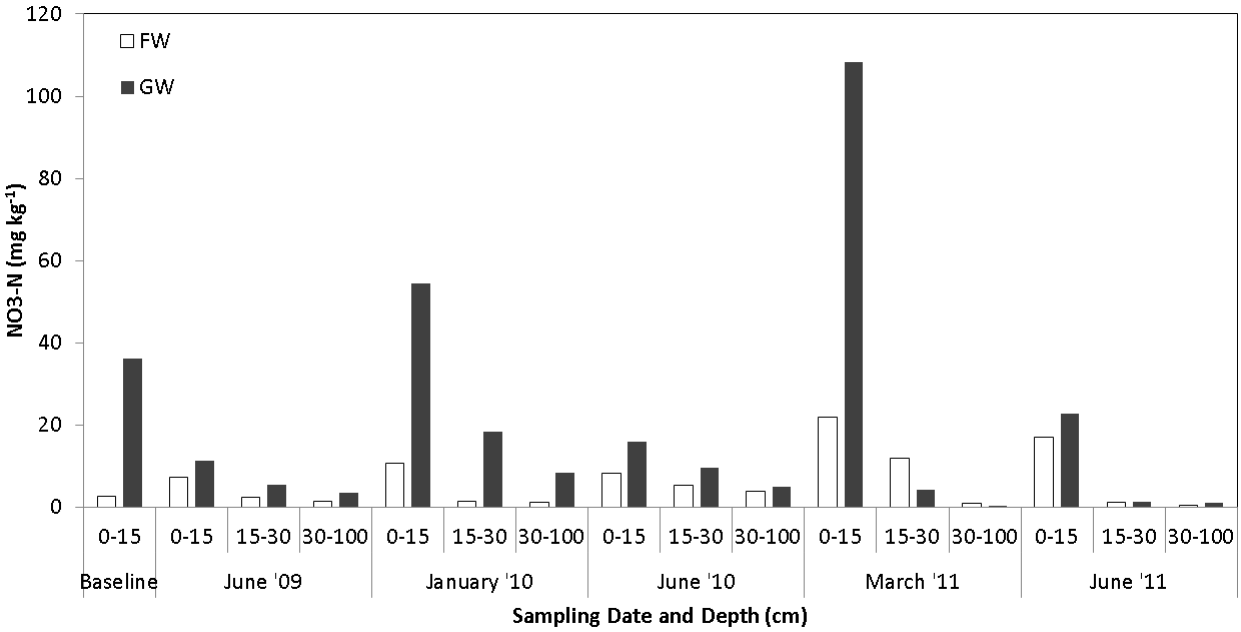

Figure 2-16. Extractable NO_3-N Measured in Soils from the AZ household with a New Graywater System, Sampled Over Time and at Various Depths.

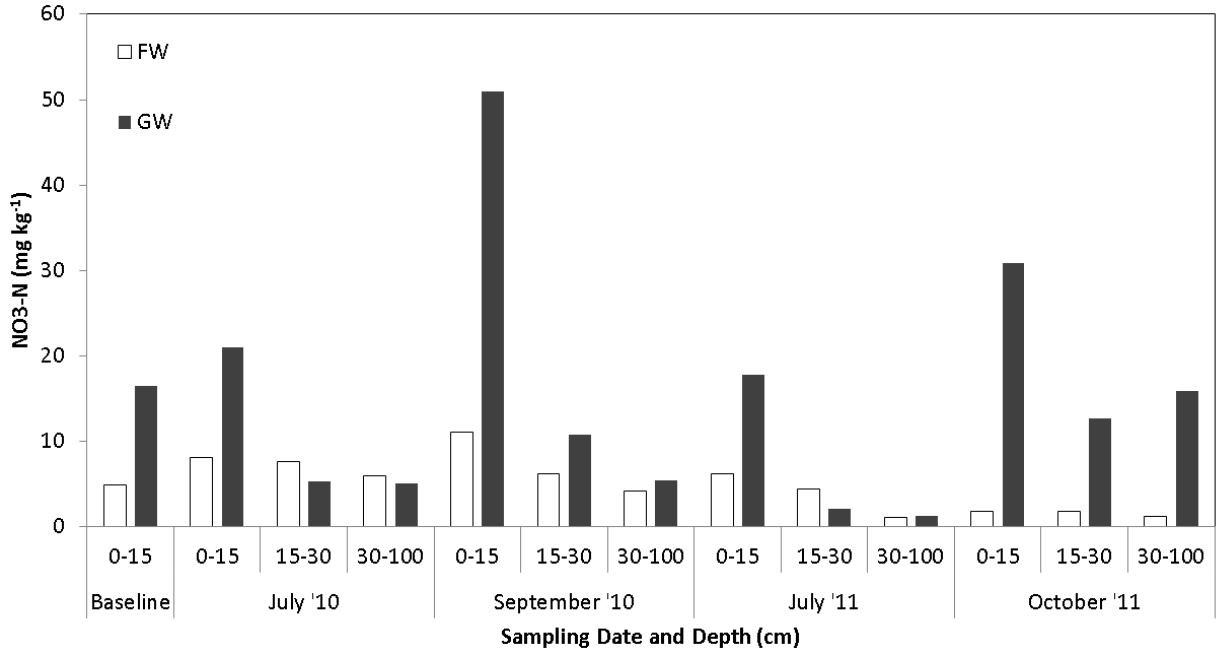

Figure 2-17. Extractable NO_3-N Measured in Soils from the CO Household with a New Graywater System, Sampled Over Time and at Various Depths (cm).

2.5.2.3 Summary

TN and TP varied among graywater and freshwater irrigated sampling locations and no consistent trend was observed for nutrients. However, 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.

2.5.3 Accumulation of Surfactants and Antimicrobials

2.5.3.1 Households with Existing Systems

Surfactants

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). Among the anionic surfactants LAS and AES and among the nonionic surfactants, AE are the most commonly used surfactants in household cleaning and personal care products. Measured concentration of LAS (C10-13), AES (C12 EO0-3), and AE (C12 EO0-9) at each household were summed to determine total surfactant concentration in surface soil (0 - 15 cm) samples collected from households with existing systems (Figure 2-16). 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. With the exception of the CA household, graywater-irrigated areas contained higher surfactant concentration than freshwater-irrigated soil samples (Figure 2-18). The CA site was highly sloped and migration of graywater into areas not irrigated by graywater was a possibility. In surface soil samples, the average total surfactants (over all sites with an existing graywater system) were 219±79 and 55±56 μmol kg^{-1} in graywater-irrigated and freshwater-irrigated soil samples respectively (data collected from CA sampling location excluded). Results indicated that graywater irrigation significantly impacted surfactant concentration in surface soil ($P \leq 0.05$). Total surfactant composition measured in surface soil samples varied at each sampling location (Figure 2-18).

Depth soil samples were collected from households in TX and CO (Figure 2-19). Total surfactant concentration decreased with soil depth in CO samples. However, at the TX sampling location, total surfactants concentration increased substantially with soil depth in the graywater-irrigated area in 2008 (Figure 2-19). This may be a result of the very high infiltration rate (Section 2.5.1.3) determined for this soil 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; 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 in addition to high infiltration capacity. In addition LAS is not readily biodegradable under anaerobic conditions which may have existed in deeper soil (Krueger et al., 1998, Scotts and Jones, 2000). This may explain the occurrence of surfactants in the deeper soil samples observed in TX. Interestingly, the increasing trend of surfactant concentration with soil depth was not observed in samples collected at the 2009 TX sampling event (Figure 2-19). 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 (78 μmol kg^{-1}). At this sampling location, LAS was the dominant surfactants measured in the depth soil samples (30-100 cm; Figure 2-19).

The risk posed by surfactants present in graywater-irrigated soil is difficult to assess. Toxicity studies have been conducted for aquatic organisms and organisms which inhabit sediment, while this data is not available for soil inhabiting organisms. The lowest no observed effect concentrations was reported for stream mesocosms at 0.22, 0.25 and 0.29 mg L^{-1} for AS, AES and LAS respectively (DK-EPA 2001). The concentration of anionic surfactants generally ranges from 4.6 to 16.7 mg L^{-1} in the graywater samples collected in this study (Table 2-7). While concentrations of surfactants in graywater are much higher than the reported no observed effect concentrations, more relevant would be to determine concentrations in soil, which would result in toxicity to organisms which typically inhabit the soil environment. This data is currently unavailable and at this time it is difficult to determine if surfactant concentrations in soil would result in toxicity to soil organisms. In addition to effects on soil ecology is the potential for phyotoxicity. Phytotoxic effects usually occur above 250 mg L^{-1} of surfactant (Bubenheim et al., 1997), well above surfactant concentration measured in graywater samples (Table 2-7). The direct phytotoxic effect will be dependent on the rate of degradation of the surfactants as well as the toxic threshold of individual plants (Garland et al., 2000). While some plants were identified to be sensitive to graywater irrigation in this study (Table 2-4), it is not possible to link this sensitivity to soil surfactant concentration.

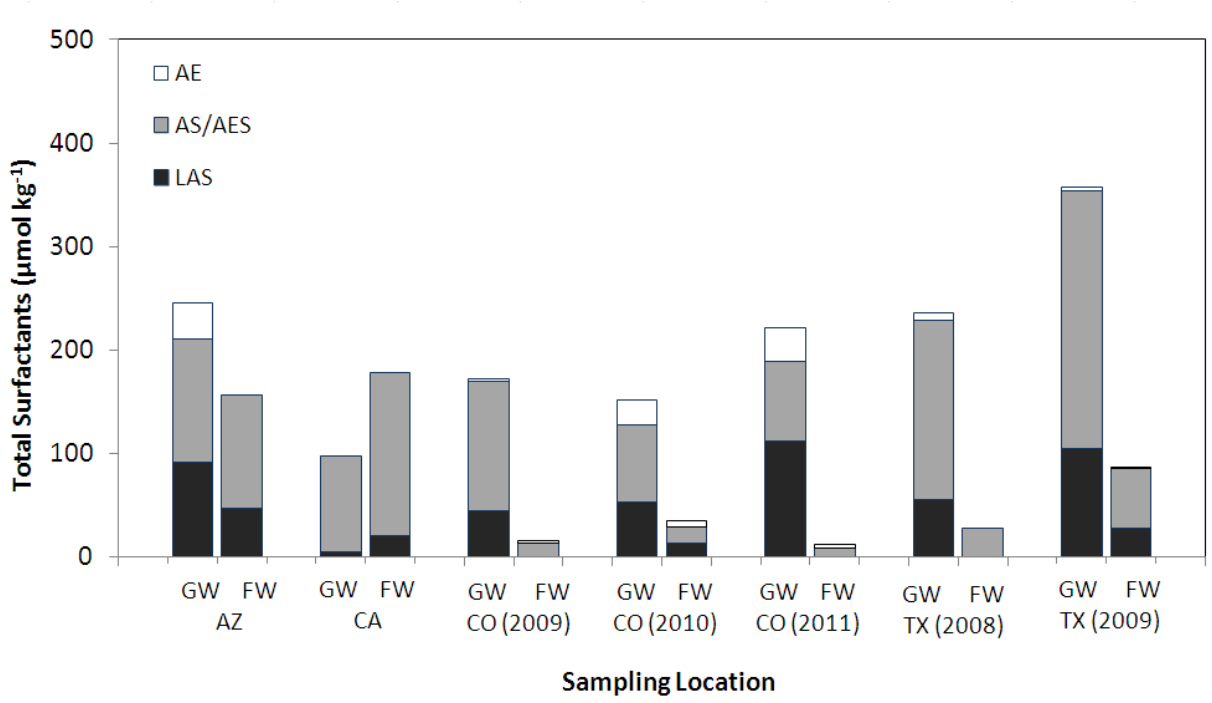

Figure 2-18. Total Surfactants in Surface Soil Samples Collected from Households with Existing Graywater Systems.

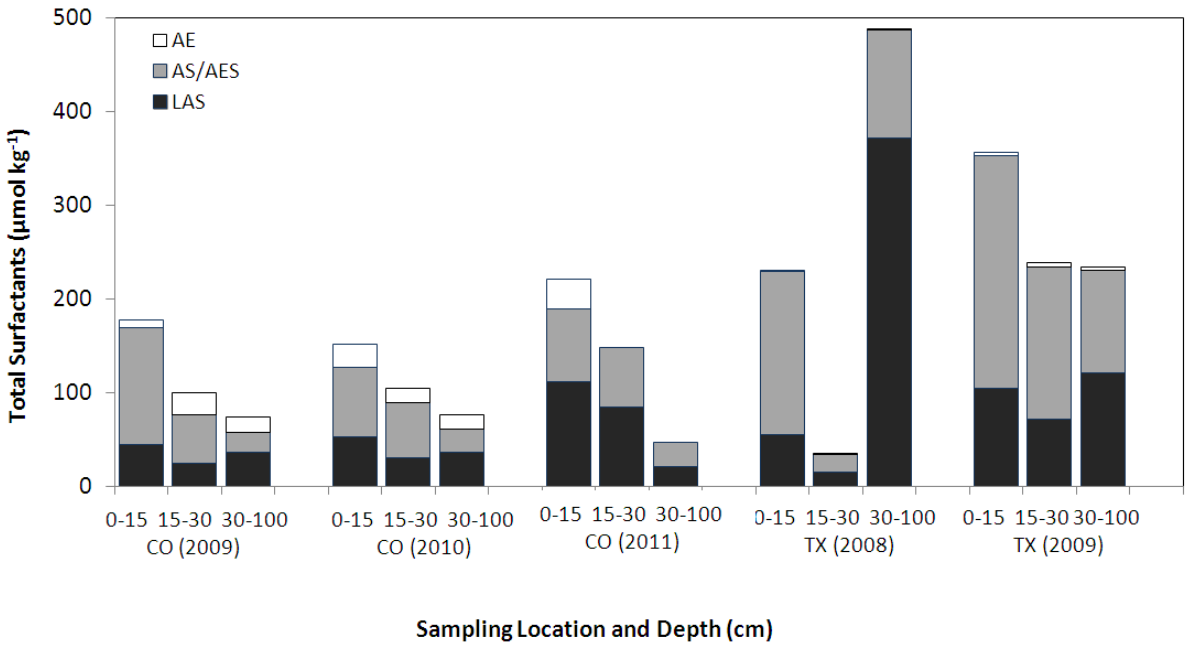

Figure 2-19. Total Surfactants in Graywater-Irrigated Depth Soil Samples Collected from CO and TX.

Fatty acid salts (soaps) are used in household cleaning products, cosmetics, lubricants (and other miscellaneous industrial applications) and coatings. Uses in household detergents and cleaning products cover chain lengths of C10-22 predominantly with counter ions of sodium and potassium. Fatty acids were analyzed in the soil samples collected from CO sampling location with existing graywater system at the last two sampling events (September 2010 and September 2011). Analyzed fatty acids in the soil samples included lauric acid, myristic acid, palmitic acid, stearic acid, palmitoleic acid, and erucic acid. Fatty acid concentrations were not notably different at this sampling location in the graywater-irrigated area compared to the freshwater-irrigated area and no significant difference was observed ($P>0.05$). In the graywater-irrigated areas fatty acids detected were 165±27, 43±10, and 28±8 mg kg^{-1} in 0-15, 15-30 and 30-100 cm soil samples respectively. In the freshwater-irrigated areas, fatty acids detected were 159±17, 41±11, and 31±7 mg kg^{-1} in 0-15, 15-30 and 30-100 cm soil samples respectively. Among the group of fatty acids measured in the soil samples, palmitic acid was the most dominant.

Antimicrobials

Two commonly used antimicrobial agents in personal care products including triclocarban (TCC) and triclosan (TCS) were measured in graywater and soil samples. Antimicrobial concentrations in surface soil samples (0-15 cm) irrigated with graywater were detected at four of the five sampling events (Table 2-8). Antimicrobials were only detected in surface soil samples and were below detection limits in depth samples. Cha and Cupples (2009) have reported concentrations of 0.05 to 1.02 µg kg^{-1} TCS and 1.20 to 65.10 µg kg^{-1} TCC in previously amended soil samples with an estimated biosolids application rate of 3.25 dry tons per acre. In surface soil receiving graywater for irrigation, the maximum observed concentration of TCS was 6.3 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 risk posed by the presence of antimicrobials in soil is difficult to

assess. 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, 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). While a predicted no-effect concentration of 115 ng L^{-1} has been derived for aquatic organisms, such toxicity studies have not been conducted for soil organisms.

Table 2-8. Antimicrobials Detected in the Surface Soil Samples. (0-15 cm; ND: not detected)

Sampling Location	TCS	TCC
	µg kg^{-1}	
AZ	3.8	6.3
CO (2009)	3.5	9.1
CO (2010)	6.3	8.4
TX (2009)	ND	2.8

2.5.3.2 Households with Newly Installed Systems

Surfactants

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 2-20 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 ($P \leq 0.05$). The trend of total surfactants in surface soil samples collected from the AZ household over the course of study was investigated (Figure 2-21). 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 reached a steady level before and after graywater irrigation seasons (Figure 2-21). 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 after termination of graywater irrigation during fall and winter (Figure 2-21).

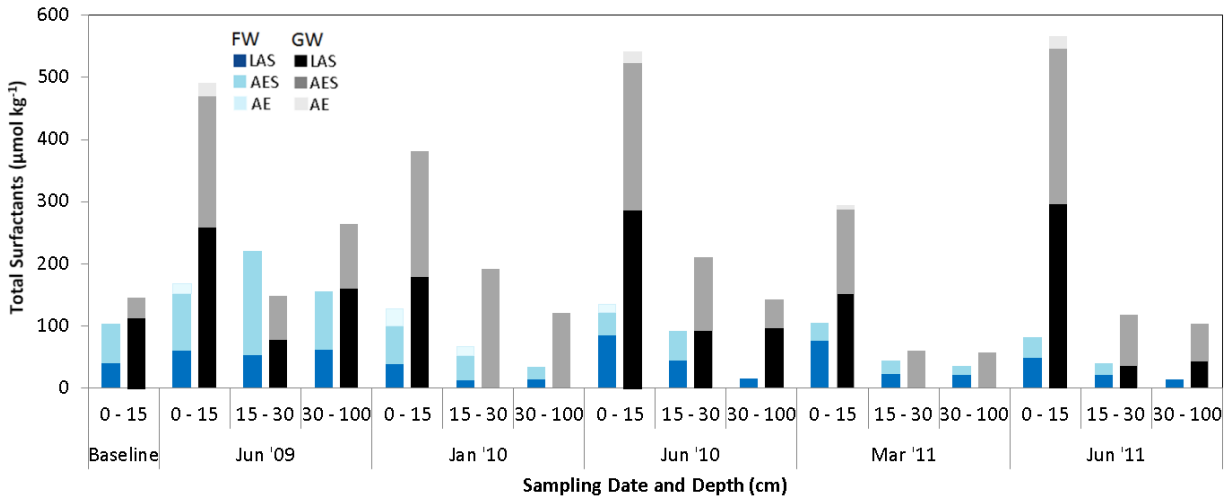

Figure 2-20. Total Surfactants in Soil Samples Collected from AZ Household with Newly Installed Graywater System.

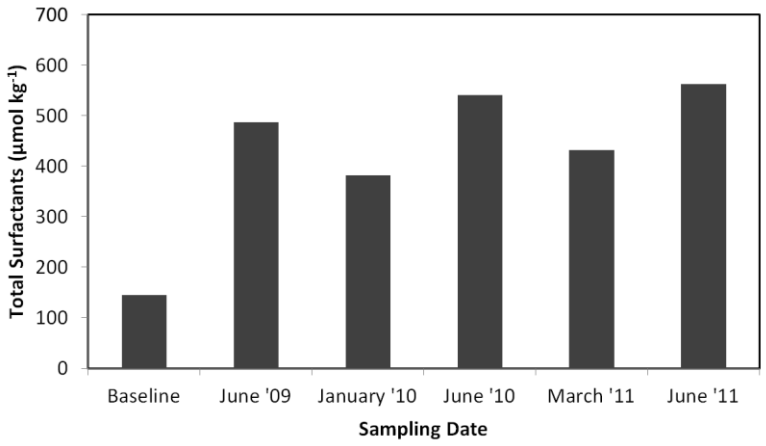

Figure 2-21.Trend of Total Surfactant in Surface Soil Samples Collected from Graywater Irrigated Soil at the AZ Household with Newly Installed System.

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 2-22). 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 2-22). Similar to AZ, surfactants increased after graywater irrigation was initiated, but did not increase notably over time.

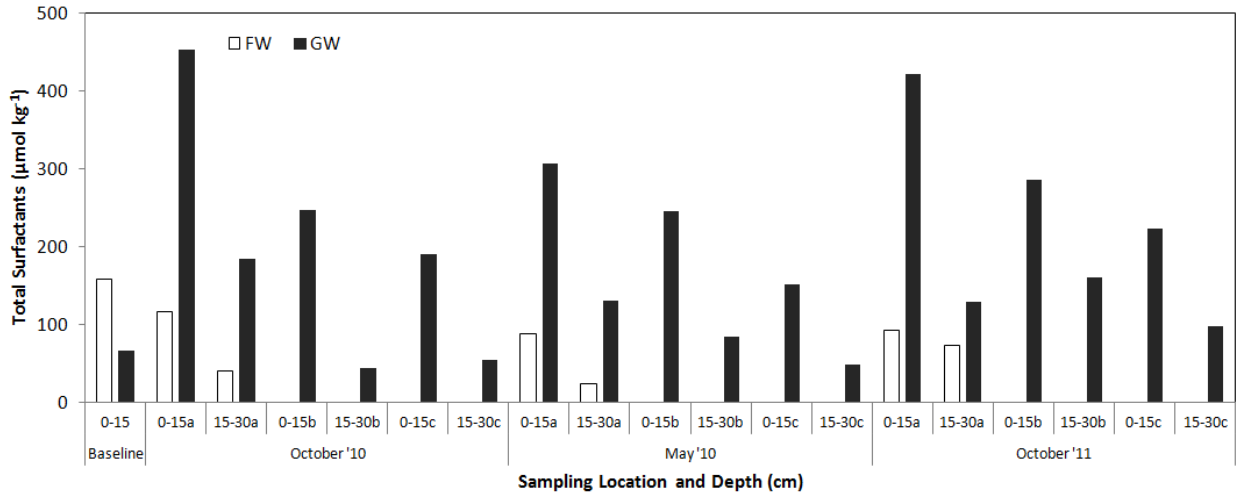

Figure 2-22.Total Surfactant in Soil Samples Collected from CA Household with Newly Installed Graywater System. (a: 0.6 m, b: 2.4 m, and c: 4.6 m distance from subsurface leach field)

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 2-23). 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. The trend of surfactant concentration in surface soil samples over time was evaluated (Figure 2-24). 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 2-24). 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 ($P \leq 0.05$).

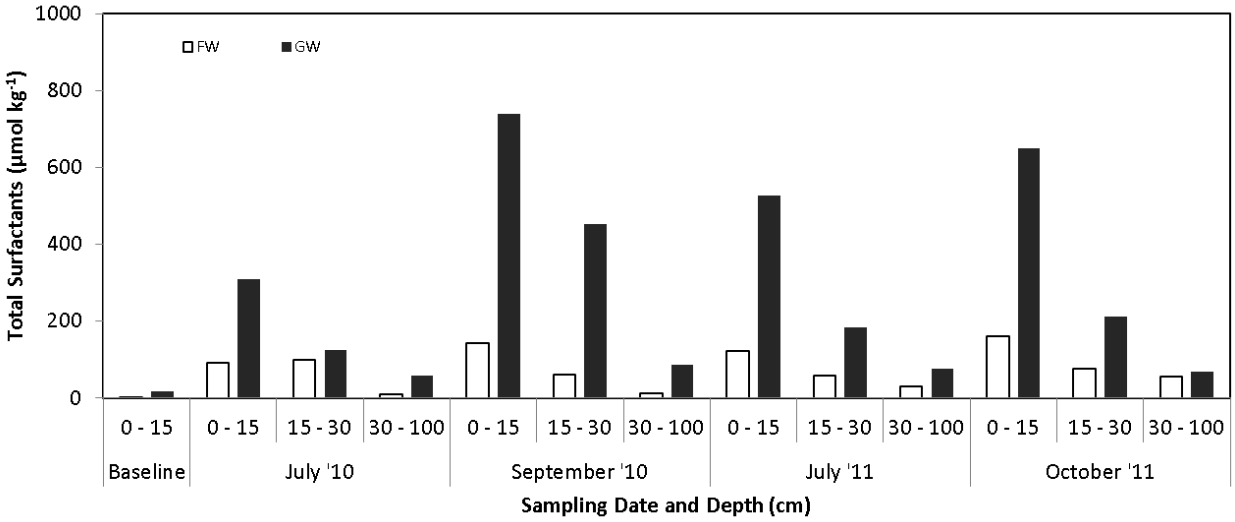

Figure 2-23.Total Surfactants in Soil Samples Collected from CO Household with Newly Installed Graywater System.

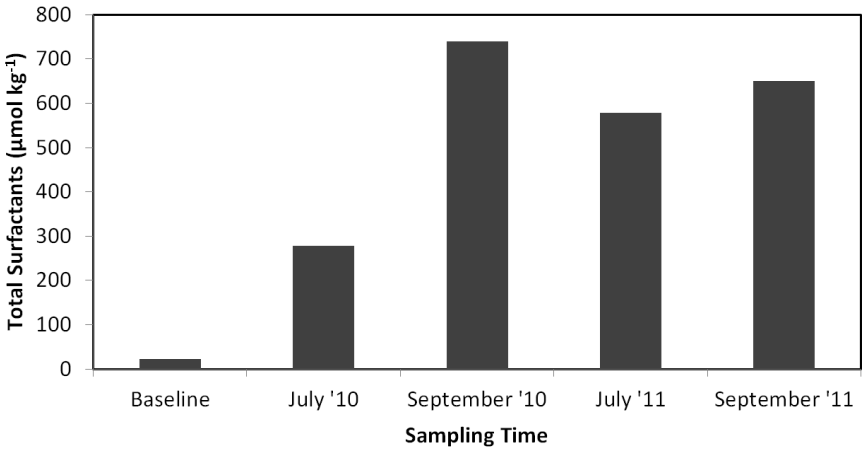

Figure 2-24.Trend of Total Surfactant in Surface Soil Samples Collected from CO Household with Newly Installed System.

The relative concentration of surfactants in graywater varied among sampling locations (Figure 2-25), indicating a difference in use of detergents and personal care products at these households. Relative ratios of AE:AS/AES:LAS were variable among all locations. AS/AES was the dominant surfactants in soil samples collected from AZ and CO location and LAS was the dominant surfactants in soil samples collected from CA respectively (Figures 2-26). The relative portion of surfactants in graywater at each household was compared to that observed in soil samples (Figures 2-25 and 2-26). 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 2-25). While at the AZ sampling site, AS/AES was the dominant surfactants in the graywater samples (79.0±4.9 %), at CA and CO sampling sites, LAS was the dominant surfactants in the graywater samples (71.4±6.6 and 70±3.7 %). 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 2-26). 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. 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.

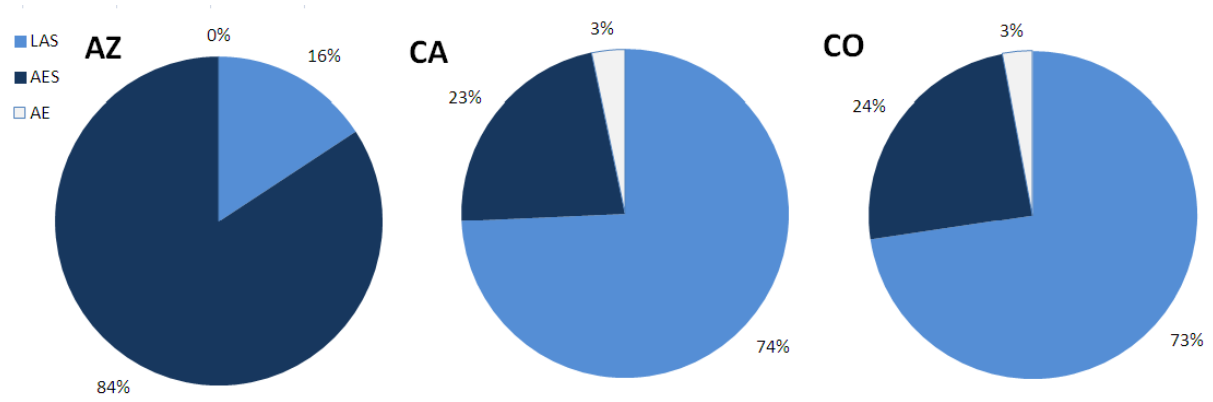

Figure 2-25. Averaged Proportion of Surfactants in Graywater Samples.

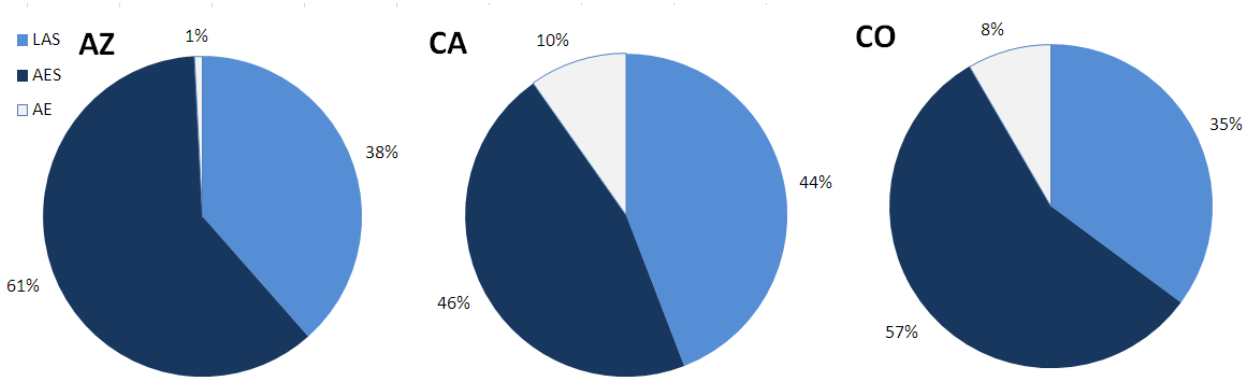

Figure 2-26. Averaged Proportion of Surfactants in Graywater-irrigated Surface Soil Samples (0-15 cm; calculated as molar mass).

Fatty acids were analyzed in soil samples collected from households with newly installed graywater systems. Analyzed fatty acids in the soil samples included lauric acid, myristic acid, palmitic acid, stearic acid, palmitoleic acid, and erucic acid. Results from analysis were summed to determine total fatty acids in the soil samples (Figure 2-27). Surface soil samples in both graywater and freshwater-irrigated areas had significantly higher fatty acids than deeper soil samples ($P \leq 0.05$). No significant difference was observed between fatty acids measured in graywater and freshwater-irrigated soil samples in AZ, CA and CO ($P > 0.05$). Among the group of fatty acids measured in the soil samples, palmitic acid and stearic acid were the two most dominant fatty acids.

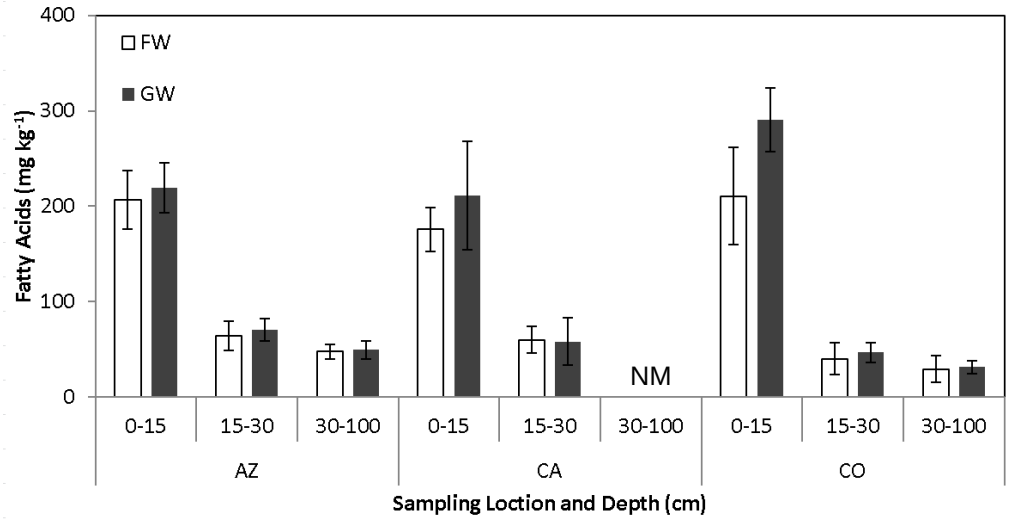

Figure 2-27.Total Fatty Acids in Soil Samples Collected from Households with Newly Installed Graywater Systems (NM: not measured).

Solubility of the different homologues of fatty acids varies but is generally estimated to be low. The C18 and C22 homologues will be insoluble at concentrations being present in the environment (HERA 2003). As a result, transport of these compounds in soil is not expected. This is consistent with results from our study, which showed significantly higher concentration of fatty acids in surface soil samples (Figure 2-27). A research project on environmental risk assessment of fatty acid salts (soap) revealed that no risk concerns were found in any environmental compartments associated with the application of fatty acids in household detergents and cleaning products (HERA 2003).

Antimicrobials

Soil samples were analyzed for antimicrobials TCS and TCC (Figure 2-28). 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).

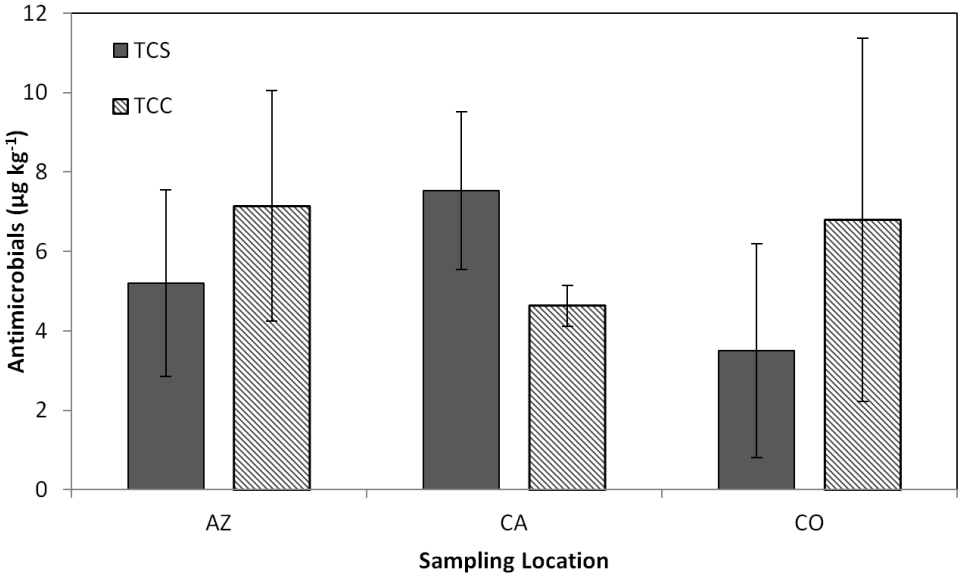

Figure 2-28.Antimicrobials Measured in Surface Soil Samples (0-15 cm) Irrigated with Graywater.

2.5.3.3 Summary

Surfactant concentration in soil collected from graywater irrigated areas was significantly higher than observed in freshwater irrigated areas ($P \leq 0.05$) at households with both existing and newly installed graywater irrigation systems. Surface soil samples collected fom graywater-irrigated areas had average total surfactants of 219±79 and 486±130 µmol kg^{-1} at sampling locations with existing and new graywater systems respectively. 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.

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. Meanwhile, in two studies (Shafran et al., 2005; Wiel-Shafran et al., 2006), up to 60 mg kg^{-1} and 30±7.2 mg kg^{-1} of anionic surfactants was detected in soil receiving graywater using the MBAS method, which is a non-specific method, much higher that than the maximum total anionic surfactant concentration observed in the current study (0.13 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 are excessively higher than those reported here because several organic 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).

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. 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.

2.5.4 Impact to Soil Infiltration Capacity

2.5.4.1 Households with Existing Systems

One concern with graywater irrigation is the decreased ability of water to infiltrate into soil over time, which may result in pooling of graywater in sites irrigated with graywater. Therefore, infiltration tests were conducted at each sampling event at the surface of the soil. Results may have been impacted by moisture content of the soil at a given sampling event. Therefore, comparisons can be made between infiltration rates in the graywater and freshwater-irrigated areas at a single sampling event, but comparisons cannot be made between different sampling events. There was no indication that long-term irrigation with graywater resulted in a substantially decreased infiltration rate (Table 2-9). While at CO and TX sampling locations, average infiltration rate in the graywater-irrigated area was consistently higher than average infiltration rate in the freshwater-irrigated area (Table 2-9), a statistically significant difference was only observed at CO sampling event in 2011 ($P \leq 0.05$; Table 2-9). Infiltration rates observed at the Texas household in 2008 and 2009 were dramatically different (Table 2-9). As previously mentioned, infiltration rates cannot be compared from different sampling events. The sampling event in 2009 was preceded by a rainfall event and soil moisture is surface samples was higer in 2009 (37.5-39.7%) compared to 2008 (25.2-28.2%). At the AZ sampling location, the freshwater-irrigated area had significantly higher infiltration rate than the graywater-irrigated area ($P \leq 0.05$; Table 2-9). Of note is that at the AZ sampling site, clay content was notably higher in the graywater-irrigated area (19%) compared to the freshwater-irrigated area (10% clay content; see Appendix C; Table C-1). In addition, OM may have contributed to the difference in infiltration rate observed at this site where OM was 1.5% in the graywater irrigated area and 5.4% in the graywater irrigated area (Appendix C; Table C-1). The difference in infiltration rates observed at the AZ household was more likely related to differences in soil quality than impacts of graywater irrigation.

Table 2-9. Infiltration Rate at households with Existing Graywater Systems. (cm hr^{-1}; GW: graywater-irrigated, FW: freshwater-irrigated; n: number of tests; a, a: no significant difference (P>0.05); a, b: significant difference between GW and FW irrigated areas (P≤0.05))

Sampling Location	Irrigation Type	Date	
		Jun '09 (n=2)	
AZ	GW	13.0±3.0 [b]	
	FW	55.9±23.4 [a]	
		Sep '10 (n=3)	Sep '11 (n=3)
CO	GW	41.7±29.7 [a]	31.8±11.9 [a]
	FW	8.6±6.1 [a]	17.3±9.4 [b]
		Sep '08 (n=1)	Oct '09 (n=3)
TX	GW	241.3	41.1±18.5 [a]
	FW	165.1	15.0±7.9 [a]

2.5.4.2 Households with Newly Installed Systems

Table 2-10 demonstrates the results of infiltration tests conducted at households with newly installed graywater systems. While at some sampling events, infiltration rates were higher in graywater-irrigated and for other sampling events higher infiltration was observed in freshwater-irrigated soil. No consistent trends were observed in terms of infiltration rates observed in graywater and freshwater-irrigated areas, and consistent with results from the households with existing systems (Section 2.5.4.1), there was no indication that long-term irrigation with graywater resulted in a substantially decreased infiltration rate.

Table 2-10. Infiltration Rate at households with Newly Installed Graywater Systems. (cm hr^{-1}; *: baseline sampling; n: number of tests; a, a: no significant difference (P>0.05), a, b: significant difference difference between GW and FW irrigated areas (P≤0.05))

Sampling Location	Irrigation	Date				
AZ		Oct '08* (n=1)	June '09 (n=2)	Jan '10 (n=2)	Jun '10 (n=2)	Mar '11 (n=3)
	GW	10.4	16.0±5.3[a]	19.8±10.9[a]	46.7±5.6[a]	7.1±7.6[a]
	FW	10.7	6.6±1.8[a]	4.3±3.6[a]	7.6±2.8[b]	17.5±19.3[a]
CA		Oct '10* (n=3)	May '11 (n=3)	Oct '11 (n=3)		
	GW	13.46±8.1[b]	10.9±1.5[a]	95.0±16.2[a]		
	FW	78.7±27.9[a]	83.8±61.0[a]	76.2±20.3[b]		
CO		Sep '09* (n=2)	Jul '10 (n=2)	Sep '10 (n=2)	Jul '11 (n=3)	Sep '11 (n=3)
	GW	43.2±3.0[b]	50.1±4.5[a]	132.1±59.7[a]	17.0±13.7$_b$	39.9±53.0[a]
	FW	101.6±4.3[a]	83.8±1.6[a]	94.0±18.0[a]	111.8±24.9[a]	99.1±4.3[a]

2.5.4.3 Summary

Results from this research indicate no long-term impact of graywater irrigation on soil infiltration capacity. 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. For example, an increase in soil sodicity can reduce water infiltration rates into soil (Oster and Shcroer, 1979). Results from this research (Table 2-8) indicate no long-term impact of graywater irrigation to soil infiltration capacity, even in Texas where graywater was applied for more than 30 years and was likely applied at a high rate due to the method of application. Also of note is that in CO where clay content was between 34-56 %, infiltration rate was consistently higher in areas irrigated by graywater.

2.6 Fecal Indicator Microorganisms

Soil microbial evaluations for all sampling locations are located in Appendix D, Tables D-1 (households with existing systems) and D-2 to D-4 (households with new systems), and results are summarized here.

2.6.1 Households with Existing Systems

E. coli was generally non-detectable from soil samples from the AZ and operating CA system households, so only data from CO and TX are further reported. All household soils were sampled to a depth of 100 cm below the ground surface. In CO, *E. coli* was detected only in the surface soil samples (0-15 cm depth) (Figure 2-29), and in 2009, *E. coli* was detected in both the freshwater- and graywater-irrigated areas, but subsequently was only detected in the graywater-irrigated area. *E. coli* results are expressed as most probably number (MPN) estimates.

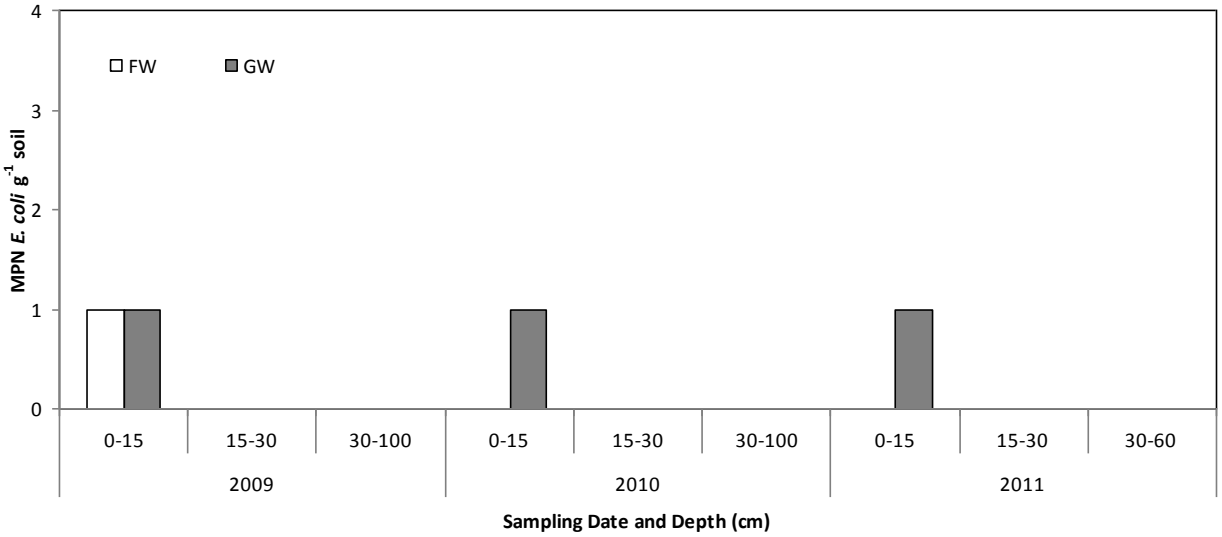

Figure 2-29. *E. coli* MPN estimates for CO Household Soils, Sampled Over Time and at Various Depths.

E. coli was detected more consistently at the TX soil site. In 2008, *E. coli* were more numerous in the graywater-irrigated area than in the freshwater irrigated area. MPN estimates were elevated in the 30-100 cm depth increment as well, indicating that *E. coli* leached from graywater-irrigated surface soils (Figure 2-30). This pattern was not observed, however, for the 2009 sampling event.

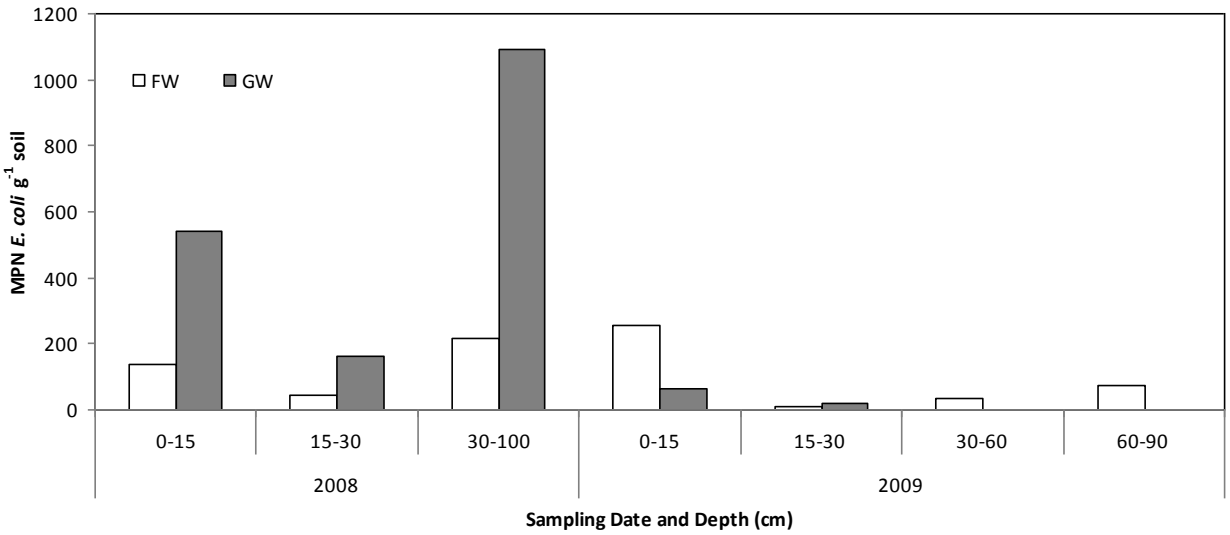

Figure 2-30. *E. coli* MPNs in TX Household Soils, Sampled Over Time and at Various Depths.

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* estimates. While *E. coli* were sometimes detected in graywater-irrigated areas, graywater irrigation did not significantly impact on *E. coli* estimates across soil locations ($P \leq 0.05$). *E. coli* were detected in potable water-irrigated areas as well as graywater-irrigated areas, and sometimes in even high numbers in potable water-irrigated areas.

Enterococci were detected in all existing household soil samples (0-15 cm depth; Figure 2-31), but there was no consistent trend between the two irrigation treatments. Enterococci were more numerous in graywater- than freshwater-irrigated surface soil only at the CA site, the CO site in 2011, and the TX site in 2008 (Figure 2-31; y-axis is log-scaled). There was no evidence of enterococci leaching through the soil profile at the CO site (Figure 2-32). In the graywater-irrigated area at the TX site, enterococci counts increased with soil depth down to 30 cm, but declined to very low values in soil deeper than 30 cm from the surface (Figure 2-33).

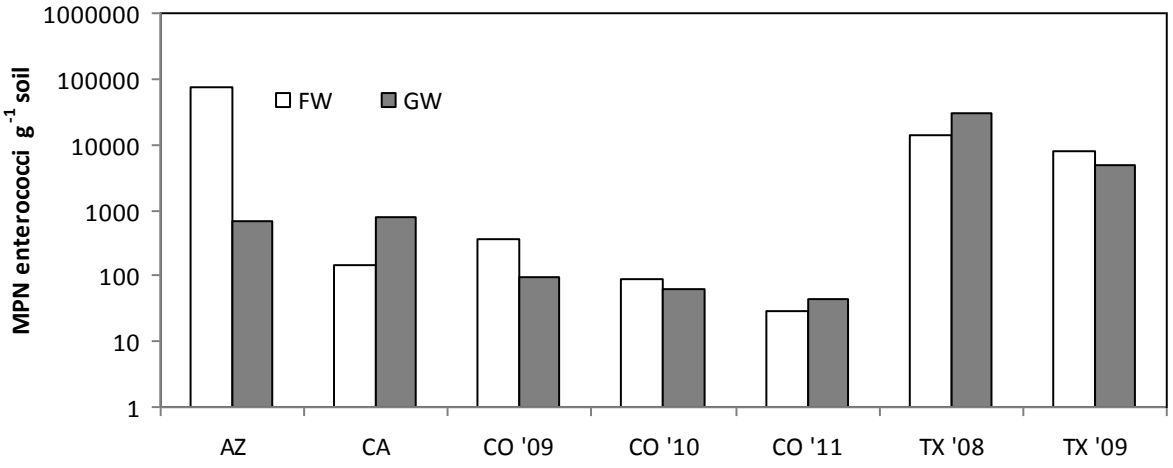

Figure 2-31. Enterococci Estimates for Household Soils (0-15 cm depth)

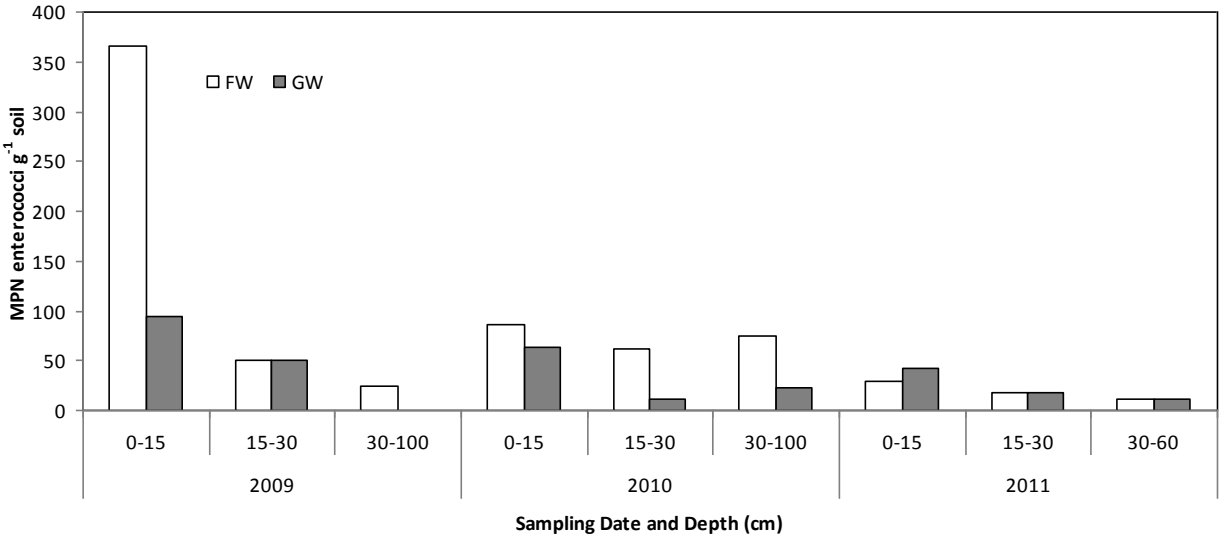

Figure 2-32. Enterococci Estimates for CO Household Soils, Sampled Over Time and at Various Depths.

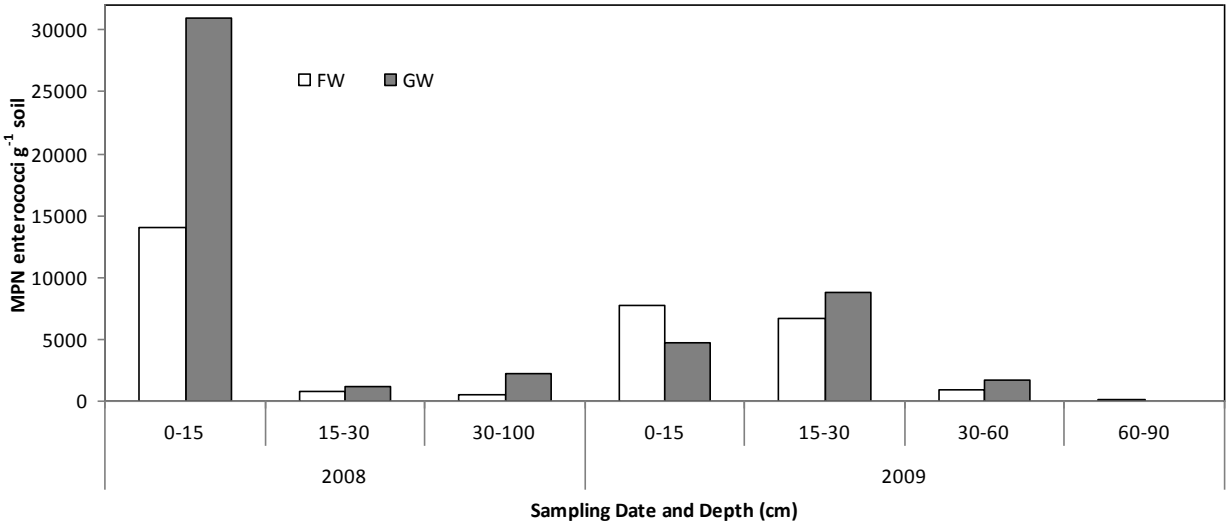

Figure 2-33. Enterococci Estimates for TX Household Soils, Sampled Over Time and at Various Depths.

2.6.2 Households with New Installations

E. coli were sporadically detected in soils from households with new graywater systems. *E. coli* were consistently more numerous in graywater-irrigated soil than freshwater-irrigated soil at the AZ site, where MPNs were concentrated within the first 15 cm of soil (Figure 2-34). However, high baseline counts in October 2008 confound the interpretation of graywater impacts at this household. In contrast, *E. coli* numbers in graywater-irrigated soil from the CA site in October 2011 (two feet from graywater irrigation line) were elevated above baseline values from September 2008 (Figure 2-35). Similarly, *E. coli* were more abundant in graywater-irrigated soil at the CO site in July 2011 compared to September 2009, when baseline samples were collected

(Figure 2-36). In addition, relatively high estimates of *E. coli* in the 15-30 and 30-100 cm depth increments at the CO site in July 2011 indicate leaching graywater-born *E. coli* from the surface.

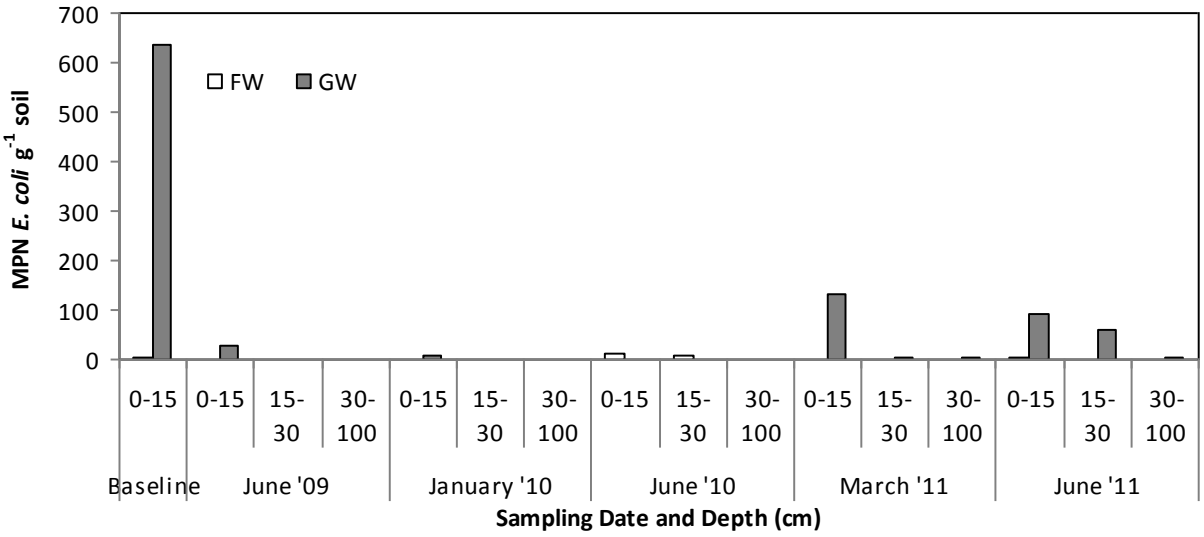

Figure 2-34. *E. coli* MPNs in Soils, Sampled Over time and at Various Depths from the Newly established AZ Household Graywater System.

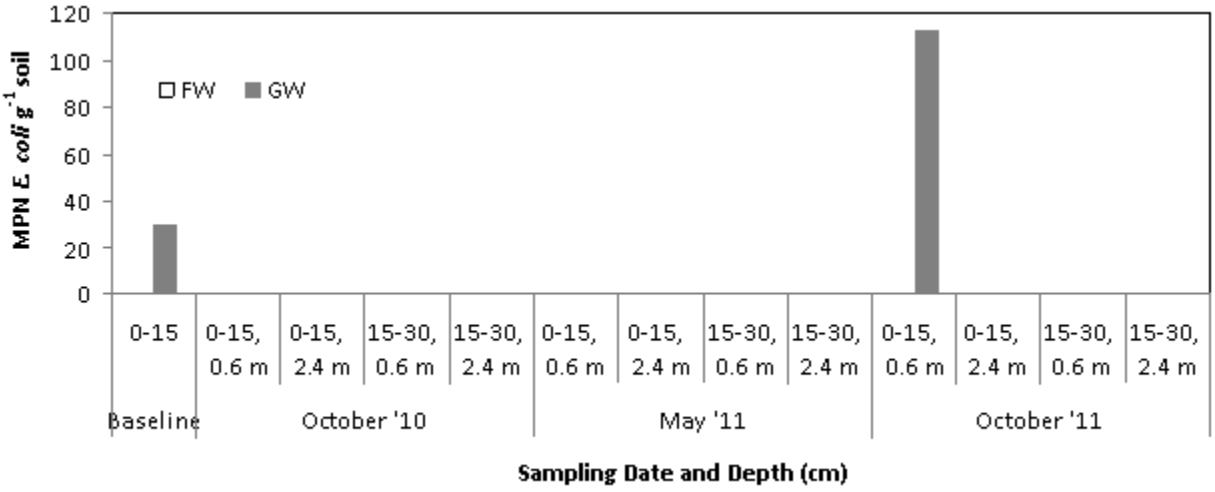

Figure 2-35. *E. coli* MPNs in Soils, Sampled Over Distance (m) and at Various Depths from the Newly Established CA Household Graywater System.

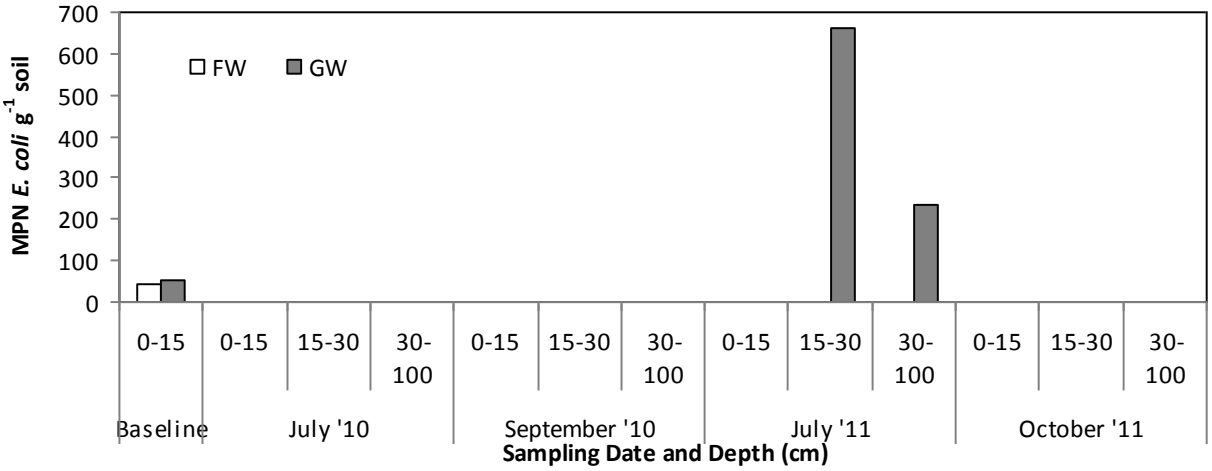

Figure 2-36. *E. coli* MPNs in Soils, Sampled over Distance (m) and at Various Depths from the CO Household with a New Graywater System.

Patterns of enterococci abundance at the AZ site were similar to that of *E. coli*, with it being difficult to interpret graywater impacts due to high baseline fecal indicator values (Figure 2-37). At the CA site, enterococci were more numerous in graywater-irrigated soils than in freshwater-irrigated soils, and numbers increased over time at distances two and eight feet from the graywater irrigation line (Figure 2-38). At the CO household, enterococci were generally more abundant in graywater-irrigated areas, but like in AZ, baseline values of enterococci were extremely high prior to the start of irrigation (Figure 2-39).

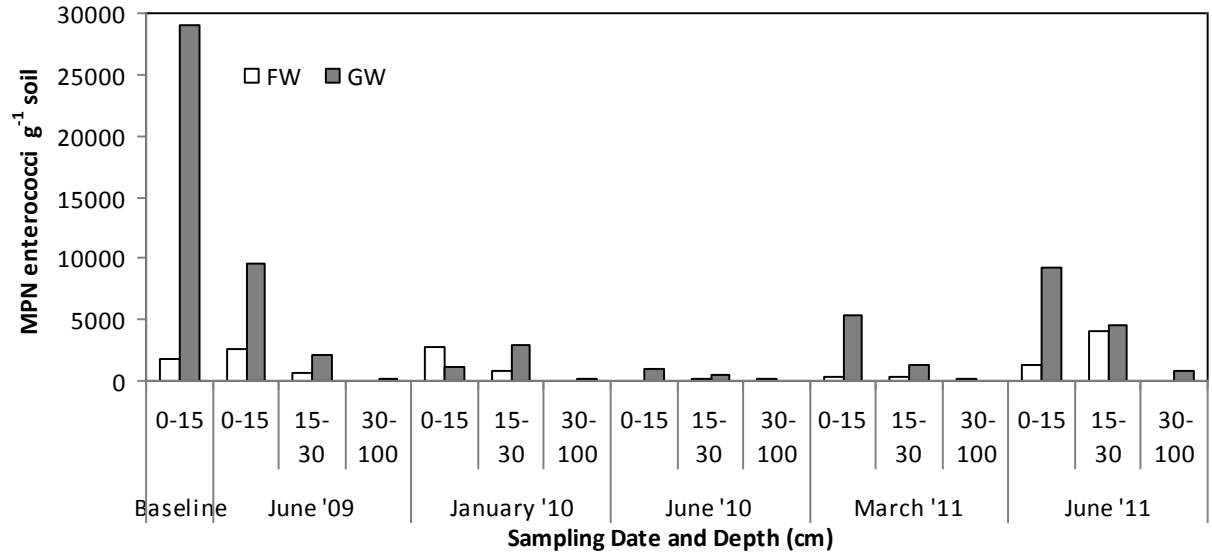

Figure 2-37. Enterococci Estimated in Soils, Sampled Over Time and at Various Depths from the AZ Household with a New Graywater System.

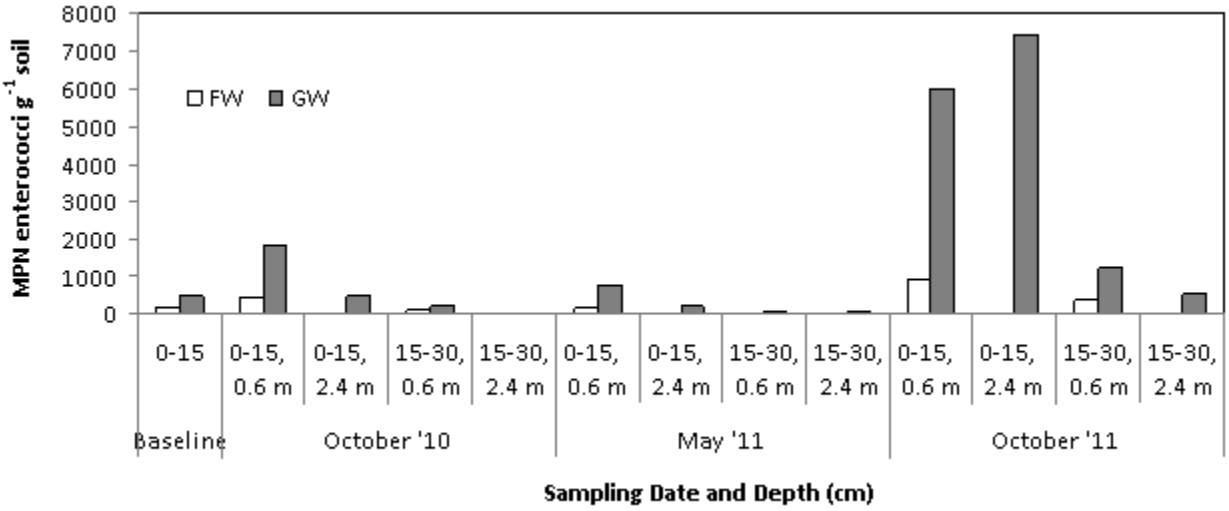

Figure 2-38. Enterococci Estimated in Soils, Sampled Over Time and at Various Depths from the AZ Household with a New Graywater System.

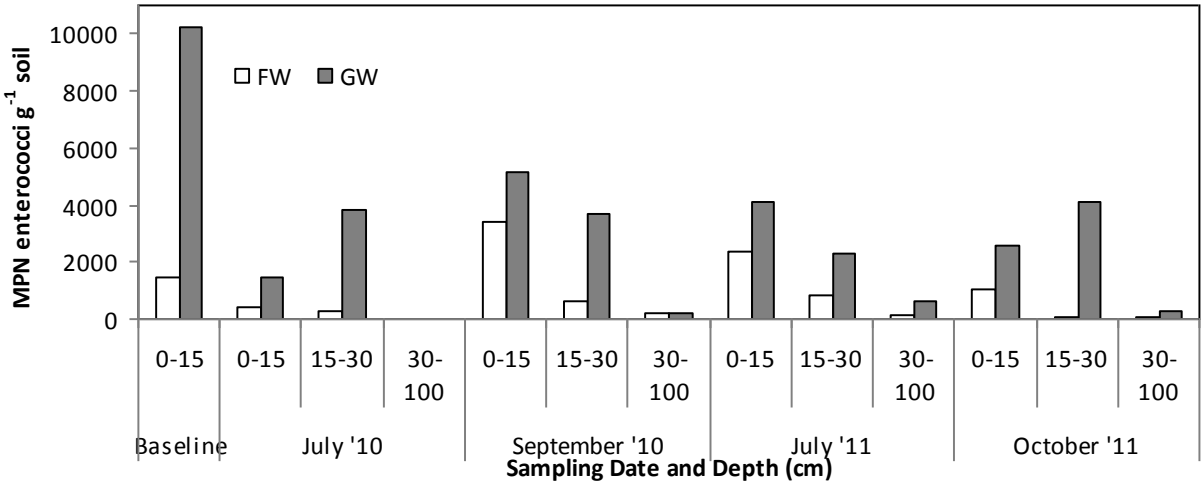

Figure 2-39. Enterococci Estimated in Soils, Sampled Over Time and at Various Depths from the AZ Household with a New Graywater System.

2.6.3 Summary

Graywater has the potential to contaminate the environment with human-associated fecal organisms, including *E. coli* and enterococci. In this study, however, the research team found no strong, consistent effect of graywater on estimates of viable *E. coli* or enterococci in soil. Contamination was inconsistent and depended on the household, sampling date, and depth of soil sampled. In addition, *E. coli* and enterococci were detected in freshwater-irrigated soils, indicating sources other than graywater for fecal indicators detected in the environment.

CHAPTER 3.0

GREENHOUSE STUDIES ON GRAYWATER IRRIGATION

3.1 Introduction

Due to difficulties that exist in determining the migration pathways and leaching of contaminants in graywater-irrigated soil through field studies, it was important to make assessments under controlled conditions. Controlled conditions eliminate of environmental effects and variability such as climatic variability, the presence of pets, and the variability in graywater application rate and composition. To remedy this problem, greenhouse experiments were set up to evaluate the possible impacts of graywater irrigation on leaching of chemical constituents in soil-plant systems. Synthetic graywater was applied instead of actual graywater to ensure consistency and repeatability of the graywater used for irrigation. Leachate was analyzed for surfactants, salts, nutrients, and other general water quality parameters. After one year of graywater application, soil was analyzed for surfactants, salts, and other physico-chemical parameters. In addition to the soil and leachate analyses, plant health evaluation was also conducted to assess the effect of graywater application on four different plant types used in the experiments.

3.2 Experiment Setup

A total of 38 custom plant pots were constructed with polyvinylchloride (PVC) pipe. The setup of the columns containing plants was a two-chamber system in which the top 55 cm contained the soil and plant biomass and the bottom 5 cm served as drainage layer (Figure 3-1). The pipes were exposed to sunlight for a period of six weeks to minimize degradation of PVC and subsequent release of toxins into plant columns during the experiments.

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 (Table 3-1). Soil was added to the plant columns and compacted to reach the bulk density of 1.5 g cm^{-3}. During the course of experiments, two types of turfgrasses and two types of shrubs were studied (Figures 3-2). The turfgrasses used were bermudagrass (a warm season grass) and tall fescue (a cool season grass). The shrubs used were meyer lemon (a citrus) and emerald gaiety euonymus (a shrub). Eight columns were planted with each plant type 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 note is that because irrigation water for the greenhouse experiment controls was potable water, it is referred to as potable water throughout Chapter 3, rather than freshwater as referred to in Chapter 2. Of the six columns without plants, three were irrigated with potable water and three were irrigated with synthetic graywater. All the plants and grasses were planted in October 2010 and were irrigated with potable water for the first five months of the study. The duration of the experiments was seventeen months from February 2010 to June 2011. Temperature was controlled at 20-25 ^{o}C in the greenhouse.

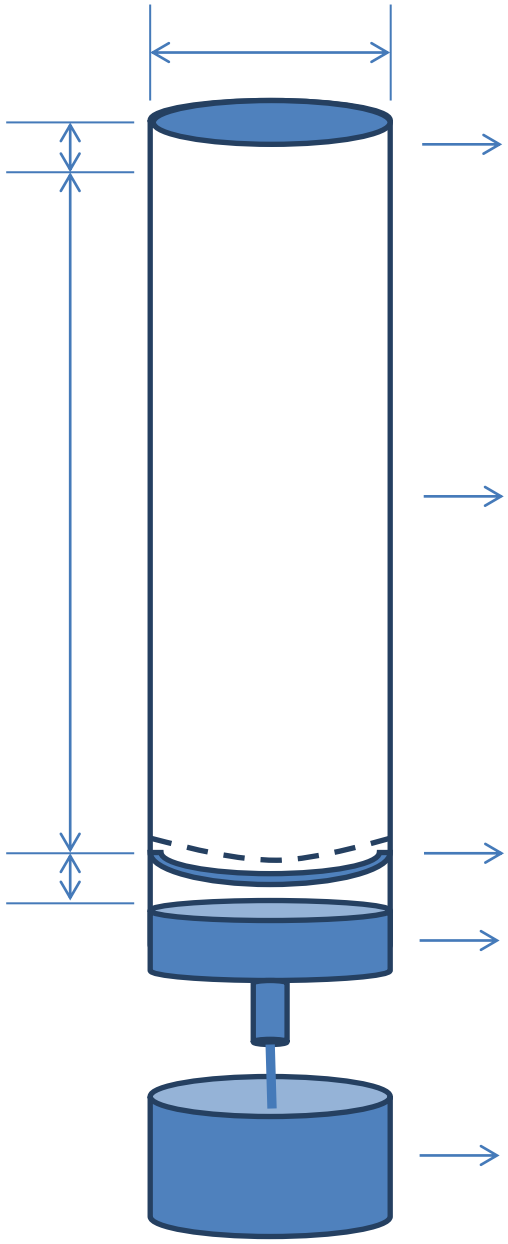

Figure 3-1. Schematic of Columns Setup.

Irrigation water was applied manually. To estimate the evapotranspiration rate in the greenhouse and subsequently determine irrigation scheduling, a bellani plate atmometer was used (Robertson and Holmes 1957). Consequently, the irrigation amount for each plant and grass was set as 120% of the plant (or grass) ET rate to ensure leachate generation. The ET for the plant (or grass) was calculated as ($ET_{plant} = coef. \times ET_{plate}$) with the appropriate plant (or grass) coefficient (coef.). Crop coefficients were adopted from Allen et al. (1998) and then adjusted to ensure enough leachate generation. Crop coefficients used in this study were 0.85, 0.95, 0.90 and 0.65 for bermudagrass, tall fescue, lemon and euonymus respectively.

Table 3-1. Soil Analysis.

Parameter		Parameter	
Sand (%)	65	CEC (meq $100g^{-1}$)	12.17
Silt (%)	17	NH_4-N (mg kg^{-1})	1.9
Clay (%)	18	NO_3-N (mg kg^{-1})	13.1
pH	7.5	TP (mg kg^{-1})	13.7
EC (µS cm^{-1})	1300	SAR	4
OM (%)	1.5	B (mg kg^{-1})	1.1
TN (%)	0.034	LAS (µg kg^{-1})	3.5
AE (µg kg^{-1})	ND	AES (µg kg^{-1})	ND

The synthetic graywater was formulated based on typical constituents found in residential graywater (Table 3-2). Because B is often found in western US soils and potentially graywater, and can have toxic effects on plant life depending on the concentration, B was also included in the synthetic graywater. Synthetic graywater was made in D.I. water on daily basis and applied immediately for irrigation in order to avoid any changes in composition. Tap water at the Colorado State University greenhouse was used for potable water irrigation and in this chapter is referred to as PW (potable water). Synthetic graywater and potable water were analyzed and compared (Table 3-3). Of note is that the synthetic graywater used in this experiment has 21 times more nitrogen (TN) and 9 times more salts (TDS) than the potable waster used. In addition,

Figure 3-2. Top Row Left to Right: Columns before Planting; Tall Fescue; Bermuda grass; Bottom Row Left to Right: Meyer Lemon; Euonymus; Setup Showing All 38 Pots.

compared to typical household graywater where SAR ranges from 2-6 (Table 2-7), the synthetic graywater used here was characterized by a low SAR of 0.8. This was a result of the complexities involved in developing a recipe to simulate graywater.

To ensure plant health, fertilizer was added to the plant columns at the initiation of the experiments. Osmocote indoor and outdoor smart-release fertilizer was used and the fertilizer amounts were adjusted for graywater-irrigated plants based on the nitrogen content of graywater used for irrigation. The fertilizer amounts of 0.32, 0.30, 0.31, and 0.35 g were added to tall fescue, bermudagrass, euonymus, and lemon columns respectively (Osmocote Technologies, Marysville, OH). For the case of potable water irrigation, the amount of fertilizer applied for all the plants and grasses was 0.47 g. The duration of the graywater irrigation was 17 months from February 2010 to June 2011. Aside from the initial fertilizer application, no supplemental fertilizer was added to any columns for the duration of the study.

Leachate generation was monitored and leachate volume was recorded during the course of the study. Leachate samples were collected in April, June and August of 2010 and January and May of 2011 from three of the four plants/grasses/no-plants irrigated with potable water and three of the four plants/grasses/no-plants irrigated with graywater. The collected samples were analyzed for various water quality parameters including dissolved organic carbon (DOC), TN, nitrate, B, SAR, conductivity, total dissolved solids (TDS), total suspended solids (TSS), volatile suspended solids (VSS), LAS, AES, AS, and AE. Methods for analysis of water, soil, and plant tissues are outlined in the QAPP delivered to WERF at the start of the project (Appendix A).

Table 3-2. Recipe of Synthetic Graywater.

Compound	Concentration (mg L^{-1})
Ammonium Chloride Crystalline	8.5
Sodium Nitrate	15.8
Sodium Borate	4.4
Calcium Chloride	47.1
Sodium Chloride	25.6
Calcium Sulfate	143.3
Sodium Sulfate	40.5
Potassium Phosphate monobasic	3.5
Magnesium Sulfate Anhydrous Powder	57.3
Potassium Chloride Crystalline	11.4
LAS (C_{12})	21.0
AES (EO_{0-3})	4.5
AE (C_{12} EO_{0-9})	0.9
Yeast Extract	248.3

At the end of experiments in June 2011, irrigation was terminated and plants were removed and analyzed for plant health evaluation. In addition, soil samples were collected from depths of 0, 25 and 45 cm from the top and soil samples were analyzed for physico-chemical parameters including OM, TN, TP, EC, pH, B, SAR, LAS, AS/AES, and AE.

Table 3-3. 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
TN (mg L^{-1})	3.88	0.18
TP (mg L^{-1})	0.8	NM
B (mg L^{-1})	0.5	≤0.1
Alkalinity (mg L^{-1} as $CaCO_3$)	158	30
SAR	0.8	NM

3.3 Leachate Production

Leachate generation was monitored and leachate volume was recorded during the course of experiments (Figure 3-3). Between October 2009 and February 2010, irrigation for both sets of columns (columns to be irrigated with graywater and the control columns) was done with potable water. In February 2010, irrigation with graywater was initiated for columns designated for synthetic graywater irrigation. Starting from March 2010, the leachate volume generated from the graywater-irrigated columns was less than that generated from the potable water-irrigated columns. The trend of lower leachate volume for the graywater-irrigated columns compared to the potable water-irrigated columns was observed for both shrubs and grasses. The difference between generated leachate collected as percentage of irrigation water from potable water-irrigated columns (42.8±18.9 %) versus graywater-irrigated columns (19.8±11.2 %) was statistically significant ($P \leq 0.05$). One cause of higher water uptake in graywater-irrigated plants may be higher growth rate of these plants. Graywater irrigated plants had significantly higher above ground biomass than potable water irrigated plants ($P \leq 0.05$; Section 3.6.1). The graywater irrigated plants had larger leaf size and canopy cover, and the grasses had a larger canopy cover as well, thus leading to larger uptake of water. 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. Less leachate was collected in late spring and summer (April through July in 2010 and March through May in 2011; Figure 3-3) due to longer daylight hours and higher plant growth, which caused higher evapotranspiration.

3.4 Leachate Quality

Leachate samples were analyzed for water quality parameters and in the interest of brevity, parameters selected for inclusion in this report include DOC, TN, SAR, EC, B, and surfactants. Of note is that insufficient leachate was generated from lemon plants for the June sampling event. This sample is noted as NM throughout graphs indicating "not measured".

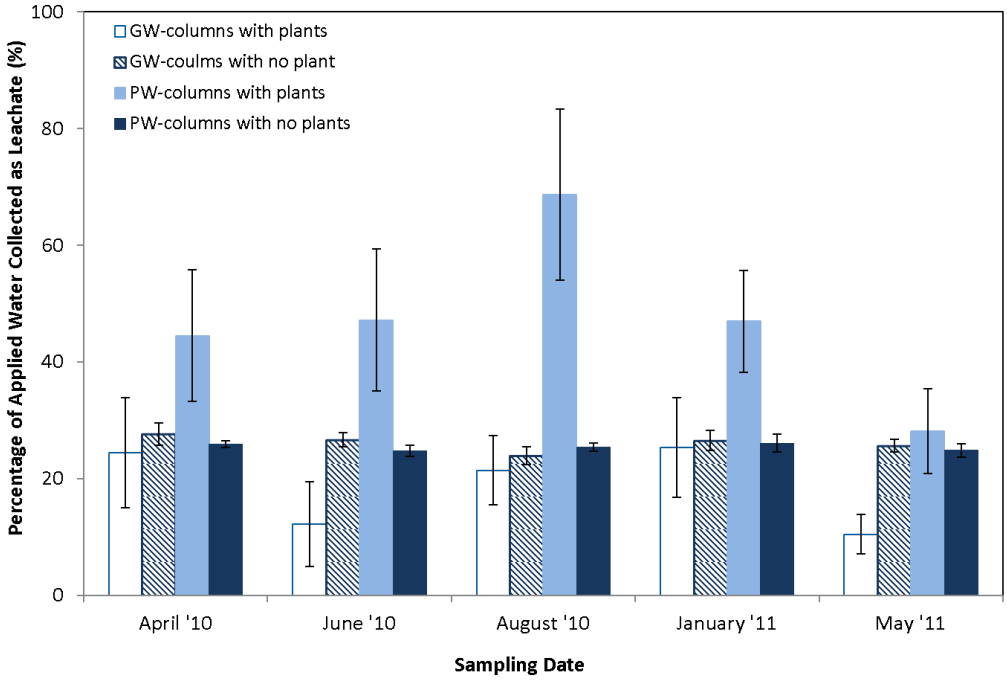

Figure 3-3. Leachate Collected as Percentage of Applied Water. (GW: Graywater-Irrigated, PW: Potable Water-Irrigated)

3.4.1 Total Salts

Leachate quality analysis (Table 3-4) revealed that TDS leached from the graywater-irrigated columns (maximum 83 mg day^{-1}) was significantly higher than TDS leached from potable water-irrigated columns (maximum of 39.7 mg day^{-1}; $P \leq 0.05$). The higher leaching of TDS was expected due to the higher input of salts from graywater irrigation compared to potable water irrigation (Table 3-3). For the January and May sampling events, TDS leached from the column containing tall fescue was slightly higher than was applied in synthetic graywater (Figure 3-4). Salt did accumulate in soils over the duration of the study (Section 3.5) and may have begun to leach out more after this accumulation. It is unclear why TDS leached from tall fescue planted columns was higher than other planted columns. Leachate quality analysis for all treatments combined, 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}; $P \leq 0.05$).

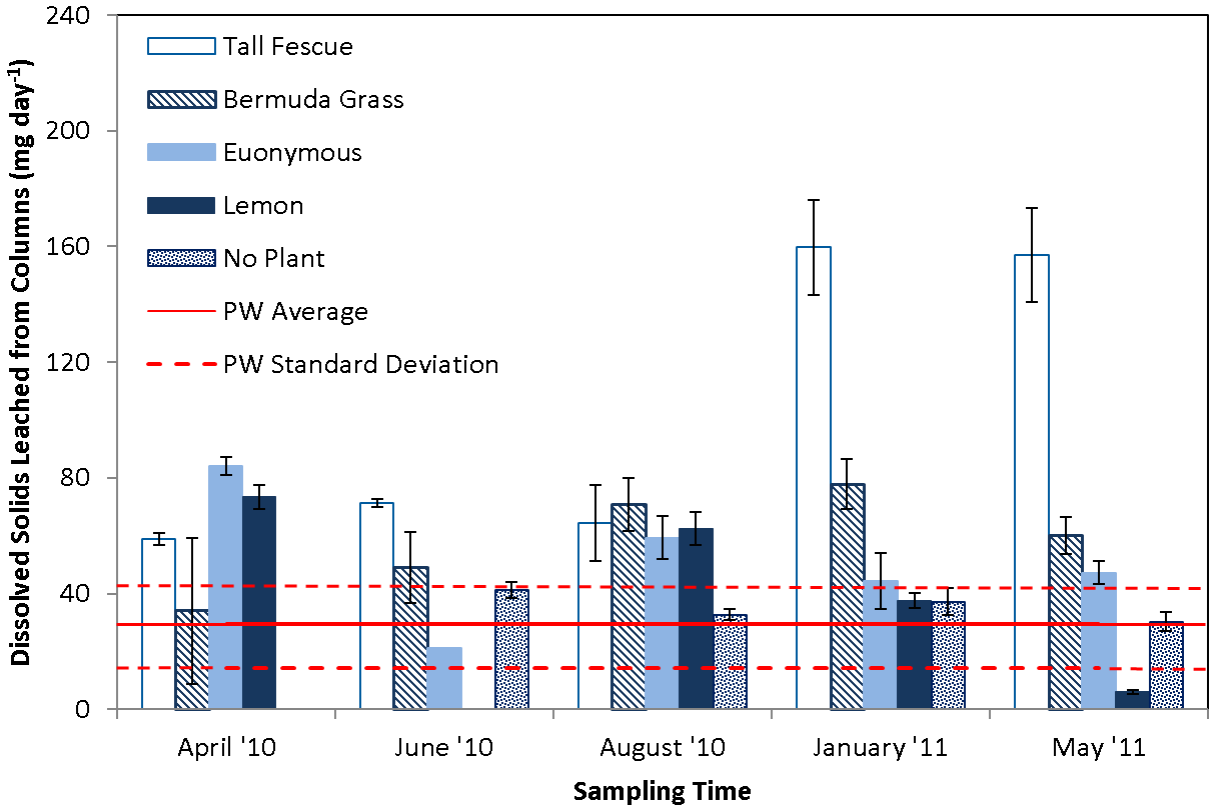

Figure 3-4. Total Dissolved Solids Leached from Columns. (average input TDS: 138±8, 98±7, 135±9, 101±7, and 107±7 mg day^{-1} for Tall fescue, bermudagrass, euonymus, lemon and no plant respectively; no leachate was generated from the lemon in June '10, so data is not reported; GW: Graywater-Irrigated, PW: Potable Water-Irrigated; PW treatments were averaged over planted columns only)

3.4.2 Nutrients

Leachate samples were analyzed for TN and TP. Of note is TP was only measured in the last two sampling events (January and May of 2011). Analysis of TN in the leachate samples revealed that significantly lower TN was leached from the columns with grass (28±7 %) than pots with shrubs (65±9 %; $P \leq 0.05$; Figure 3-5). No significant trend was observed for percentage TN leached from the columns over the duration of the study (R^2=0.34, P>0.05). Except for the first sampling event (April '10), TN leached from the columns irrigated with graywater was always significantly higher than that leached from columns irrigated with potable water (Table 3-4; $P \leq 0.05$). TN leached from unplanted columns irrigated with graywater was significantly higher than columns with plants ($P \leq 0.05$). In addition, TN leached from unplanted graywater-irrigated columns increased from 1.14±0.19 mg day^{-1} in June '10 to 1.75±0.05 mg day^{-1}in May '11. There is potential for N applied in graywater to leach through soil when applied for irrigation.

Table 3-4. Constituents Leached from Columns Irrigated with Graywater (GW) and Potable Water (PW) Where Leached Mass Was Averaged Over All Columns. (n=3; a, b: significantly different; a, a: not significantly different; NM-not measured)

Parameter (mg day^{-1})	April '10		June '10		August '10		January '11		May '11	
	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]
TN (mg day^{-1})	0.13 [a]	0.13 [a]	0.33 [a]	0.11 [b]	0.30 [a]	0.16 [b]	0.44 [a]	0.18 [b]	0.38 [a]	0.08 [b]
TP (mg day^{-1})	NM	NM	NM	NM	NM	NM	0.15 [a]	0.09 [a]	0.11 [a]	0.10 [a]
SAR	0.10 [a]	0.11 [a]	0.07 [a]	0.09 [a]	0.08 [a]	0.06 [a]	0.11 [a]	0.04 [b]	0.08 [a]	0.03 [b]

Averaged TP percentage retained in the planted columns from the last two sampling events was 79±8%, 56±5% and 54±9% for turfgrasses, shrubs and no plants respectively. No significant difference was observed in the TP leached from graywater and potable water-irrigated columns (P>0.05; Table 3-4).

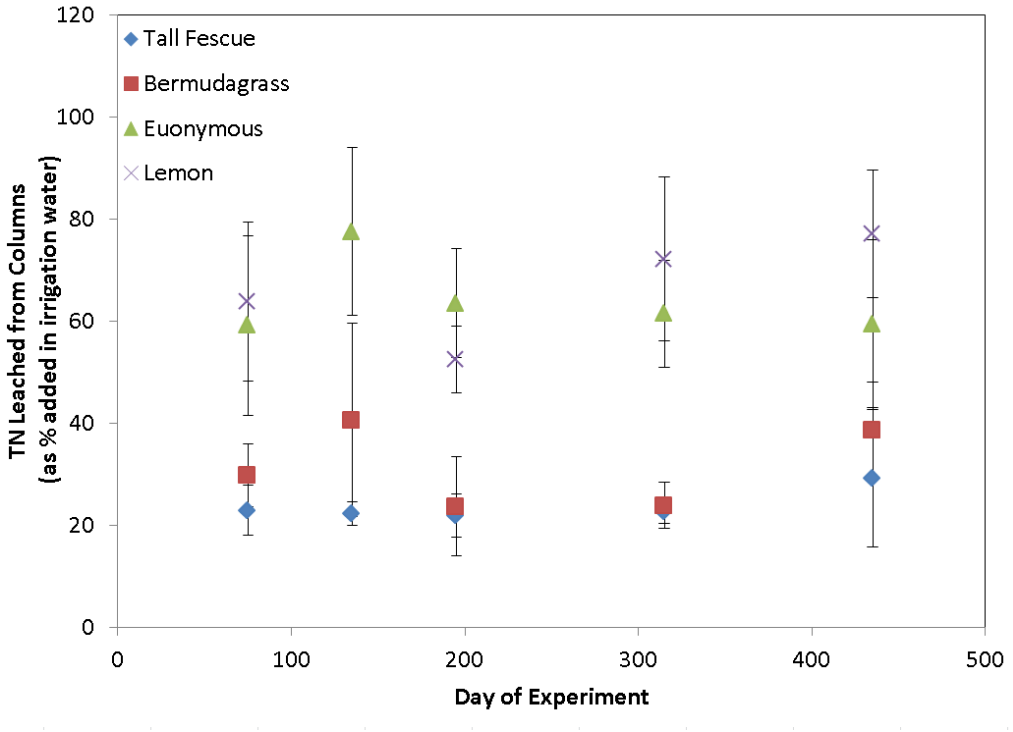

Figure 3-5. Percentage of TN Leached from the Graywater-Irrigated Columns. (no leachate was generated from the lemon in June '10 sampling events, so data is not reported)

3.4.3 Boron (B)

B was measured in all of the leachate samples. B leached from columns irrigated with graywater compared to columns irrigated with potable water was significantly higher in the last

three sampling events (Table 3-4; P≤0.05). A trend analysis was conducted on B leached from graywater-irrigated columns over the course of the study. Result showed a statistically significant increasing trend in B leached from the graywater-irrigated columns over the duration of the study (R^2=0.84; P≤0.05). Soil analysis showed that B was higher in soil irrigated with graywater than potable water (Section 3.5). It appears that as B accumulated on soil, it began to leach at a higher rate out of the columns. Through the course of the study, B did not leach at a higher rate than applied in graywater (Figure 3-6).

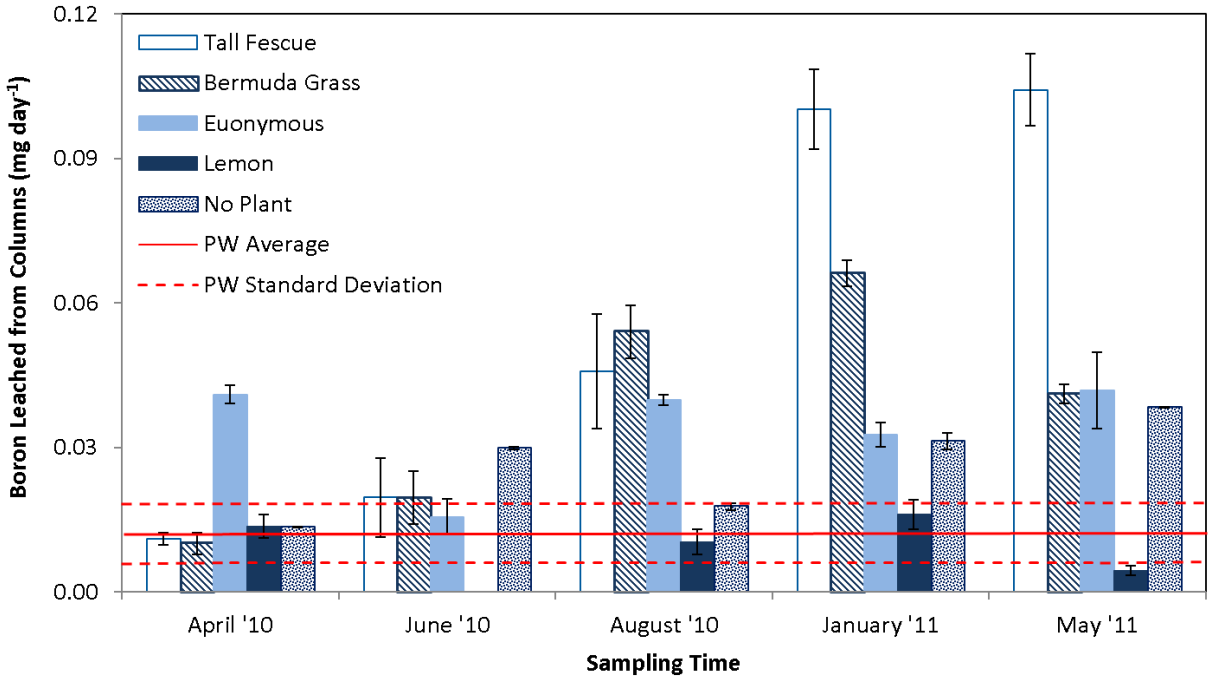

Figure 3-6. B Leached From Columns. (average input B was 0.11, 0.08, 0.11, 0.083 and 0.088 mg/day for tall fescue, bermudagrass, euonymus, lemon and no plant respectively; no leachate was generated from the lemon in June '10 sampling event, so data is not reported; GW: Graywater-irrigated, PW: Potable water-irrigated; PW treatments were averaged over planted columns)

3.4.4 Dissolved Organic Carbon (DOC)

Leached DOC values ranged from 0.11 to 2.42 mg day^{-1}, far below the input values of DOC which ranged from 13.6 to 19.8 mg day^{-1} (Figure 3-7). DOC leached from graywater-irrigated columns was not significantly different (P>0.05) than potable water-irrigated columns except for the June '10 sampling event where DOC leached from the potable water-irrigated columns was higher than the graywater-irrigated columns (Table 3-4). Given that organic input to the graywater-irrigated columns (COD 378 mg L^{-1}; Table 3-3) was much higher than potable water-irrigated columns, results indicated high retention or bioconversion of organics in the graywater-irrigated columns.

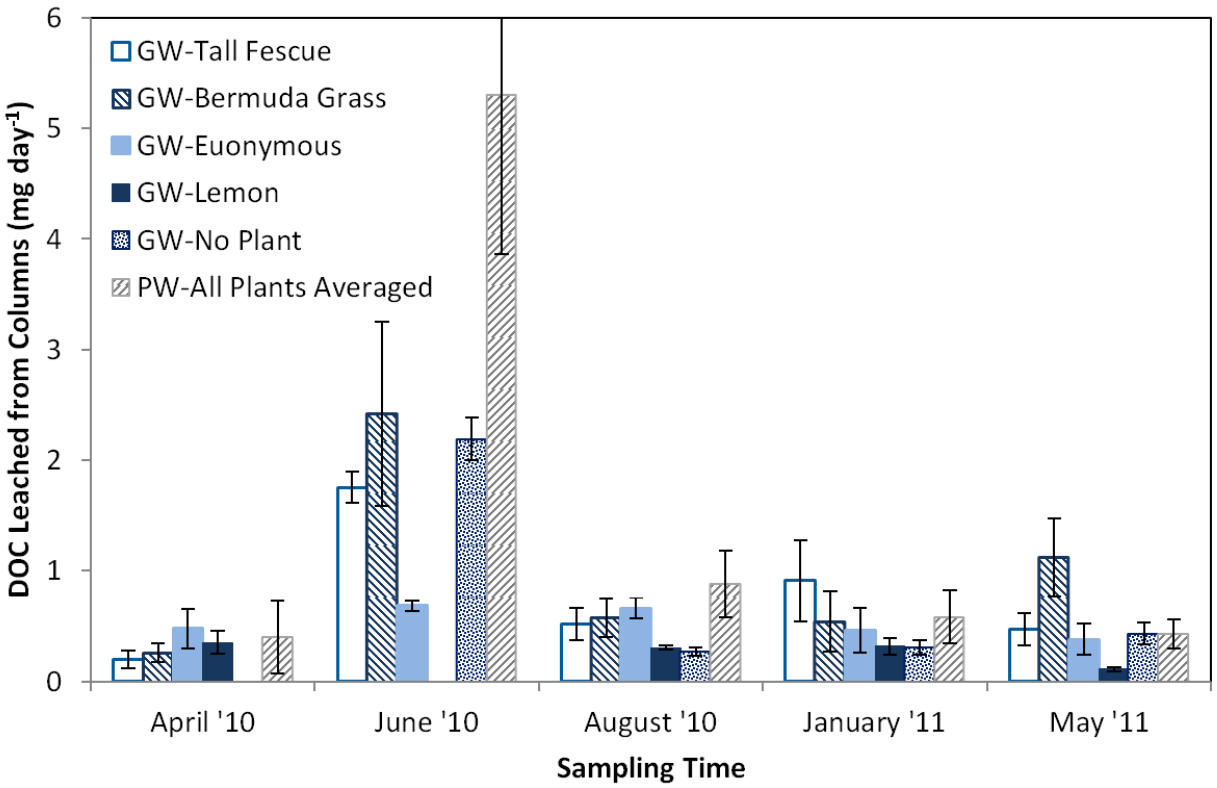

Figure 3-7. DOC Leached From Columns. (average input DOC for graywater-irrigated columns was 19.8, 13.6, 18.8, 14.1 and 14.9 mg/day for tall fescue, Bermuda grass, euonymus, lemon and no plant respectively; no leachate was generated from the lemon in June '10, so data is not reported; GW: Graywater-irrigated, PW: Potable water-irrigated)

3.4.5 Surfactants

Leachate samples were analyzed for LAS, AS/AES, and AE and values were summed to determine total surfactant concentration in the samples. Less than 17% of the surfactants added to the columns leached through the columns (Figure 3-8). Results indicated that even after 17 months of continuous irrigation with synthetic graywater, a large portion of surfactants are retained in the soil, either through adsorption or biodegradation. However, average total surfactant leached through columns as percent by mass ranged from 3% to 17% over the duration of study (Figure 3-8). A linear regression trend analysis was conducted on average retained total surfactants concentration in the columns. Results showed a statistically significant decreasing trend in total surfactants percentage retained in the columns (planted and unplanted) over the period of the study (R^2=0.86; $P \leq 0.05$). This indicates that surfactant leaching through graywater-irrigated soil may increase over time. Further study is warranted to make conclusions on the risk associated with this phenomenon. Of note is that the average total surfactant concentration in the leachate at the last sampling event (May of 2011) was 11.3±3.5 mM.

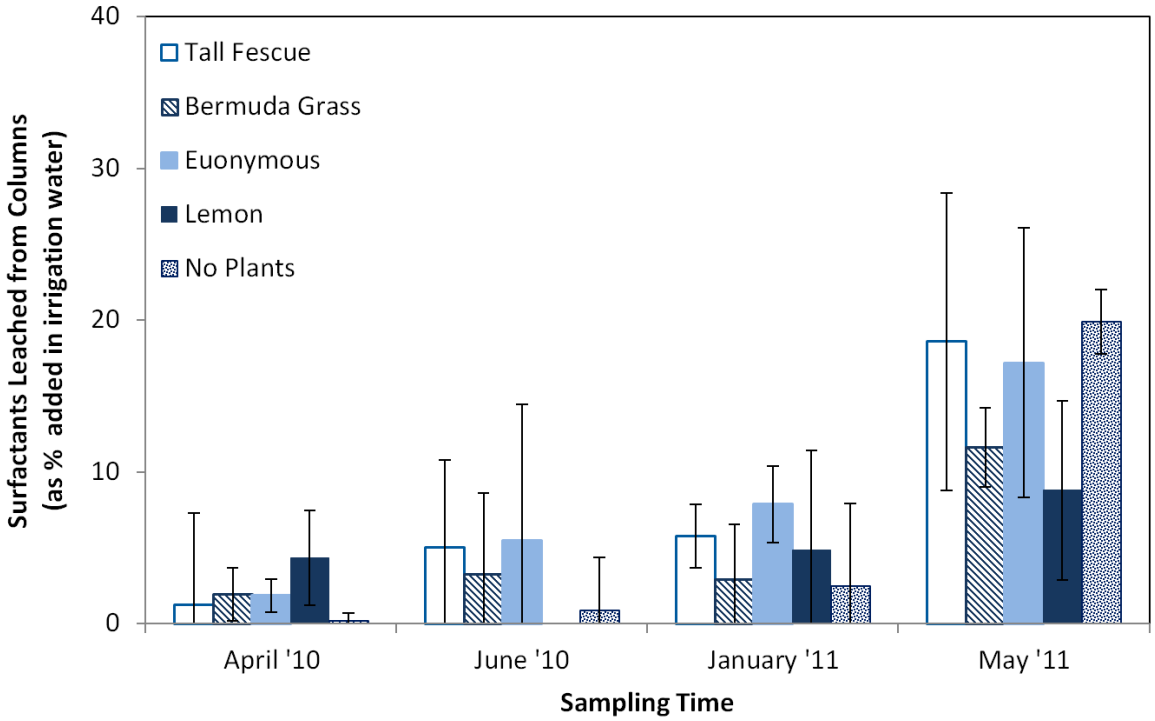

Figure 3-8. Percentage of Total Surfactants Leached from the Graywater-Irrigated Columns. (No sample was collected from lemon columns in June 2010)

3.5 Soil Quality

3.5.1 Total Salts and B

EC measured in graywater-irrigated soil samples (557±238 µS cm^{-1}) was significantly higher than that measured in potable water-irrigated soil samples (219±60 µS cm^{-1}; Figure 3-9; $P \leq 0.05$). Of note is that higher EC values were detected in deeper soil samples (45 cm) than surface soil samples (0 and 25 cm) in grass columns irrigated with graywater (Figure 3-9). This in conjunction with elevated TDS leached from graywater-irrigated columns (Section 2.4) may raise concern over the potential leaching of salts to groundwater after long-term application of graywater, or negative impact on plants.

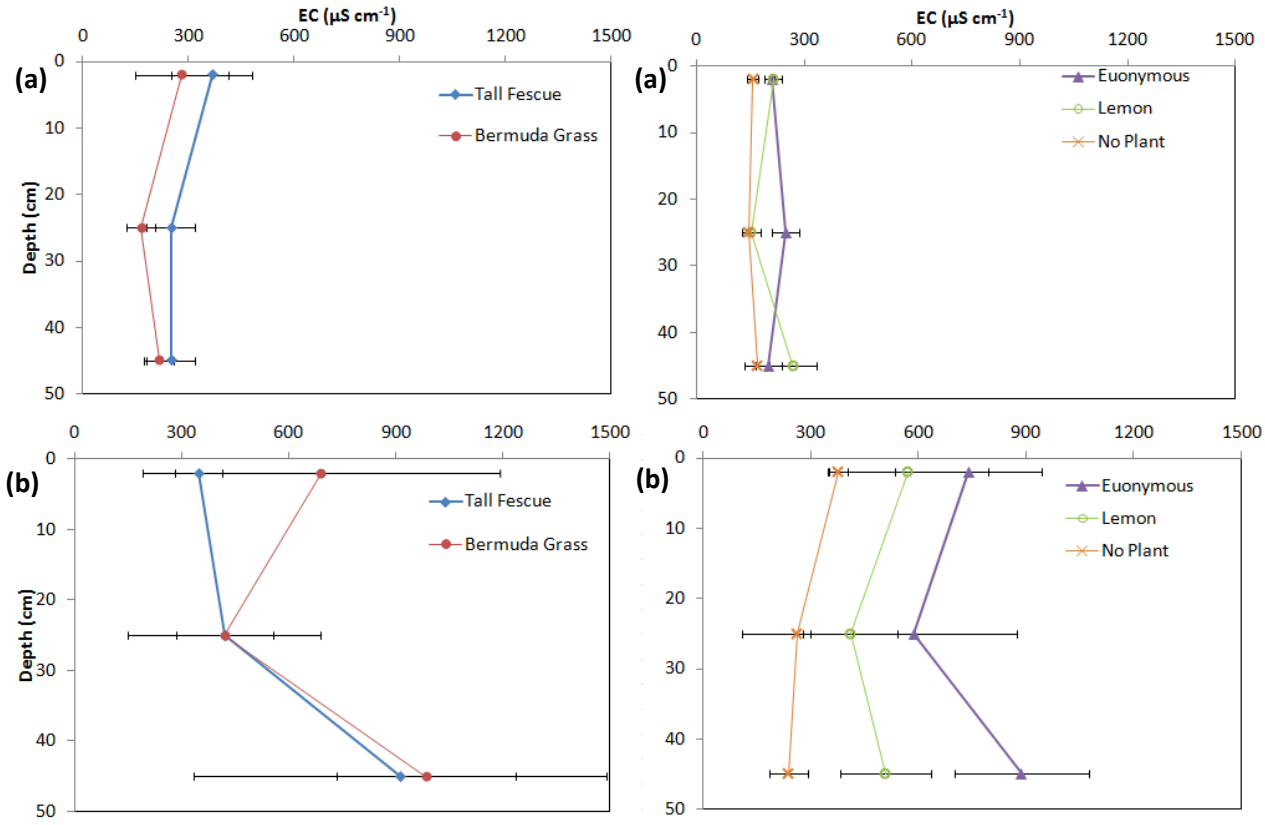

Figure 3-9. Electrical Conductivity of Soil Samples; (a) Potable Water-Irrigated, (b) Graywater-Irrigated.

Soil analysis for SAR revealed that graywater-irrigated soil samples had significantly higher SAR values than potable water-irrigated soil samples (Figure 3-10; $P \leq 0.05$). 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 graywater-irrigated soil samples was far below 5, regardless of plant type (Figure 3-10). 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, the average SAR observed in field samples irrigated with graywater for more than five years was 1.3 ± 1.1 (Section 2.5).

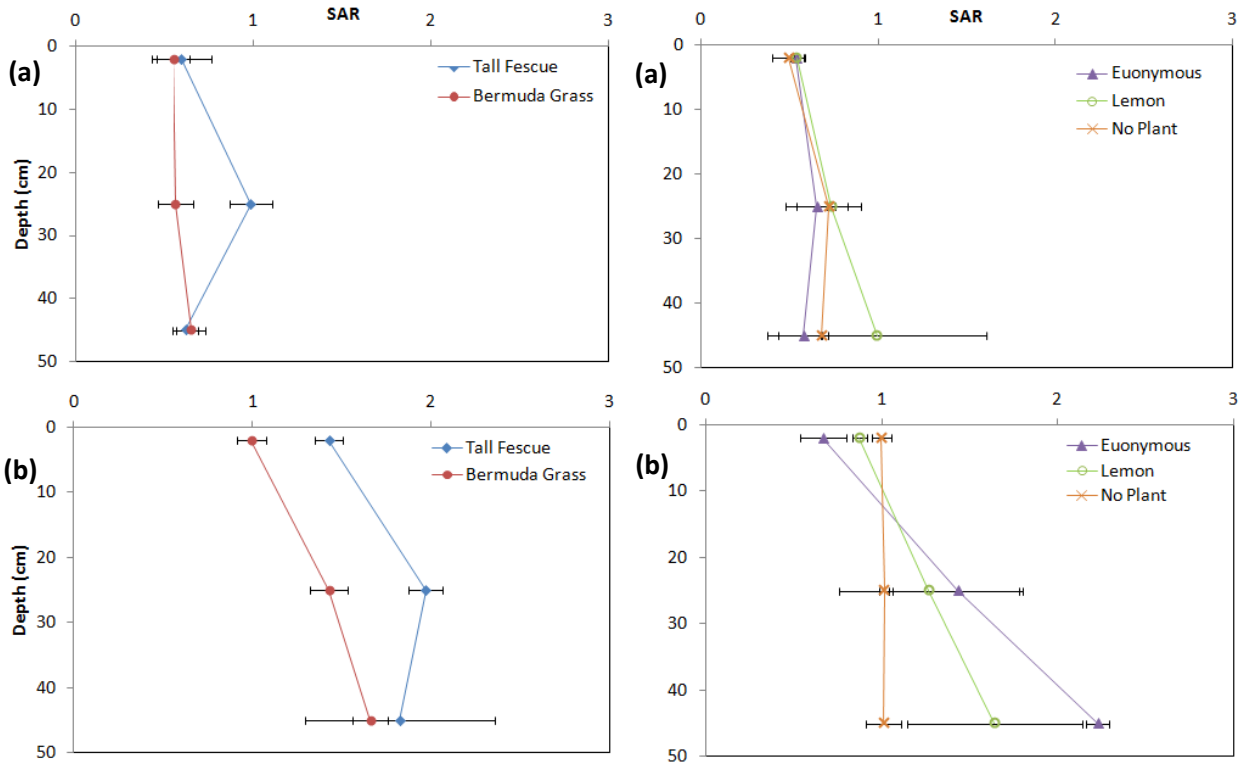

Figure 3-10. Soil Sample SAR; (a) Potable Water-Irrigated, (b) Graywater-Irrigated.

Soil samples were also analyzed for hot water extractable B (Figure 3-11). The graywater-irrigated soil samples had significantly higher B than potable water-irrigated soil samples for all columns ($P \leq 0.05$), which confirmed accumulation of B in graywater-irrigated soil. However, results from greenhouse study revealed that after 17 months of continuous irrigation with synthetic graywater, hot water extractable B in soil samples were still below the deteriorative level of 5 mg kg^{-1} (Max. 2.9 mg kg^{-1}; Figure 3-11).

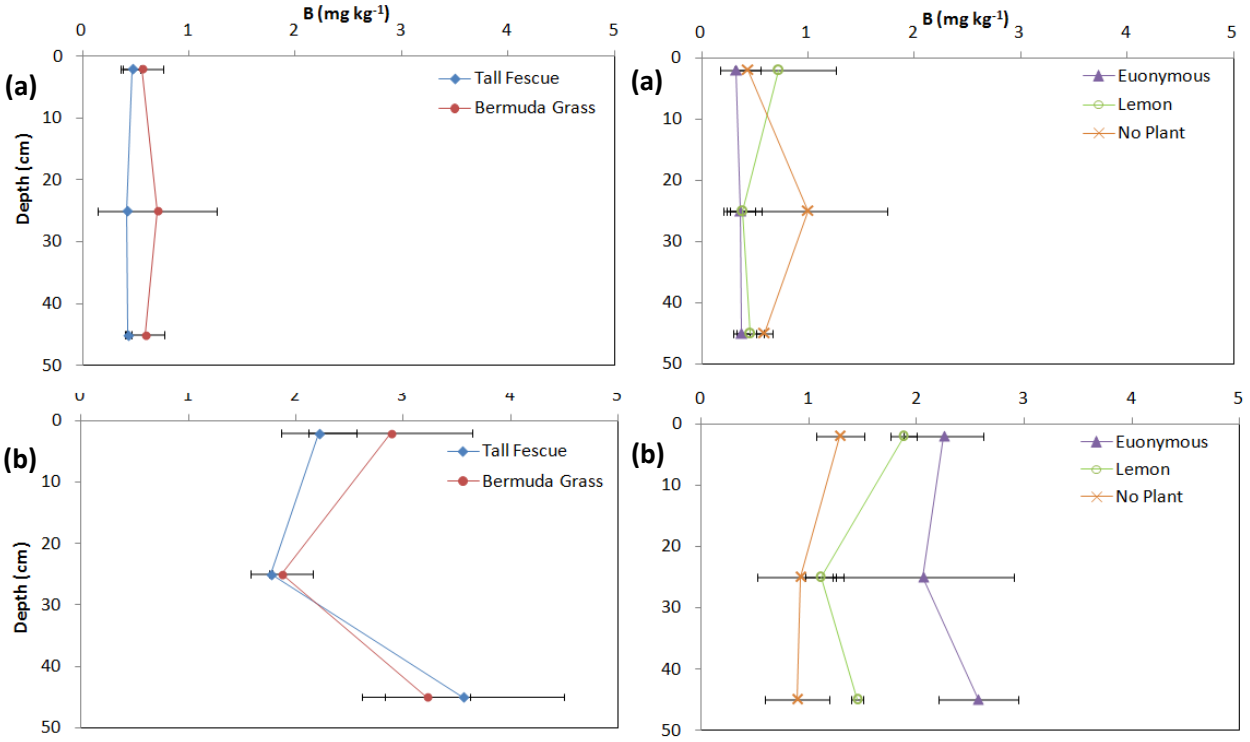

Figure 3-11. B in Soil Samples; (a) Potable Water-Irrigated, (b) Graywater-Irrigated.

3.5.2 Nutrients

Graywater-irrigated soil samples collected from 0 and 25 cm had significantly higher TN values than potable water-irrigated soil samples (Figure 3-12; $P \leq 0.05$). Results indicated that graywater irrigation resulted in accumulation of TN in surface soil samples (Figure 3-12). Results also indicated that when plants were present, there was no significant difference between TN values measured in the deeper samples (45 cm) collected from graywater and potable water-irrigated pots ($P > 0.05$). Surface soil samples irrigated with graywater contained up to five times more TN than those irrigated with potable water, indicating an excess of nitrogen which could serve as a source for increased biomass of graywater irrigated plants (see discussion in Section 3.6).

TP was analyzed in soil samples. Except in surface soil samples collected from euonymus and unplanted pots, no significant difference was observed in TP measured in soil samples collected from graywater versus potable water-irrigated columns (Figure 3-13). No accumulation of TP was observed in graywater-irrigated columns (Figure 3-13). TP in synthetic graywater was low (0.8 mg L^{-1}; Table 3-3) and did not result in substantial accumulation of TP in graywater-irrigated soil.

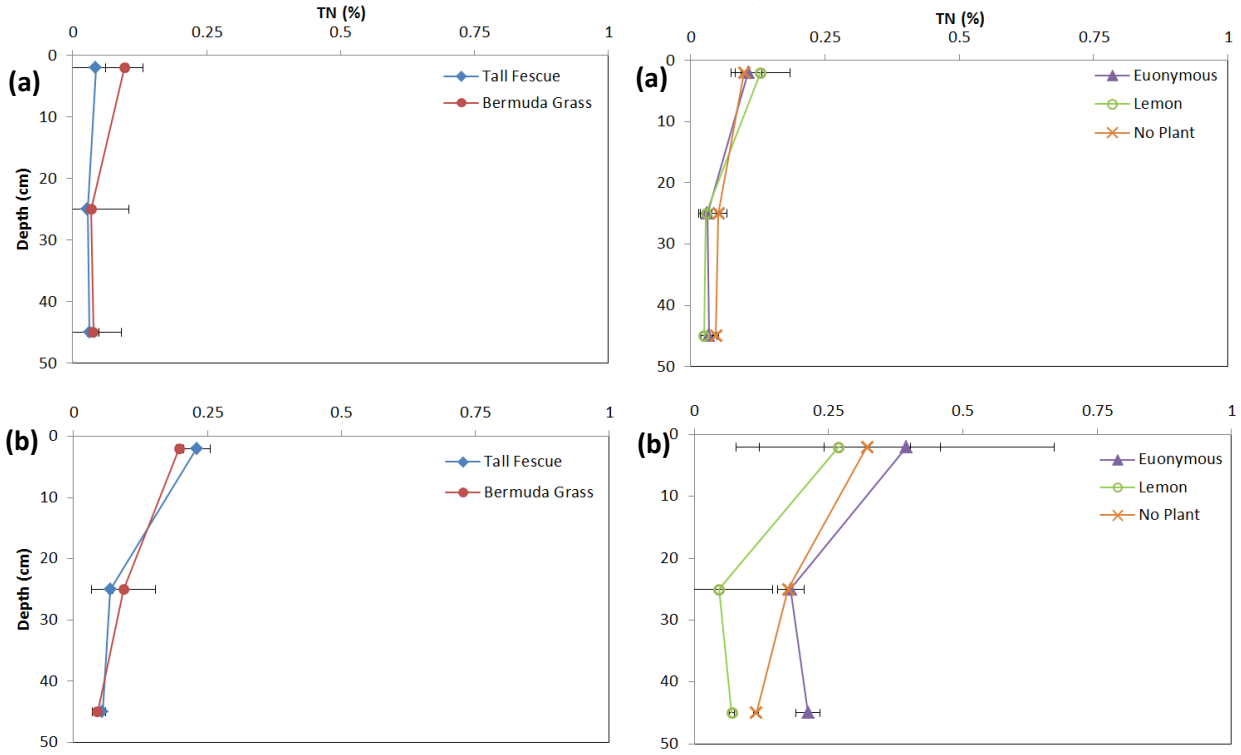

Figure 3-12. TN Measured in Soil Samples in Different Depths; (a) Potable Water-Irrigated, (b) Graywater-Irrigated.

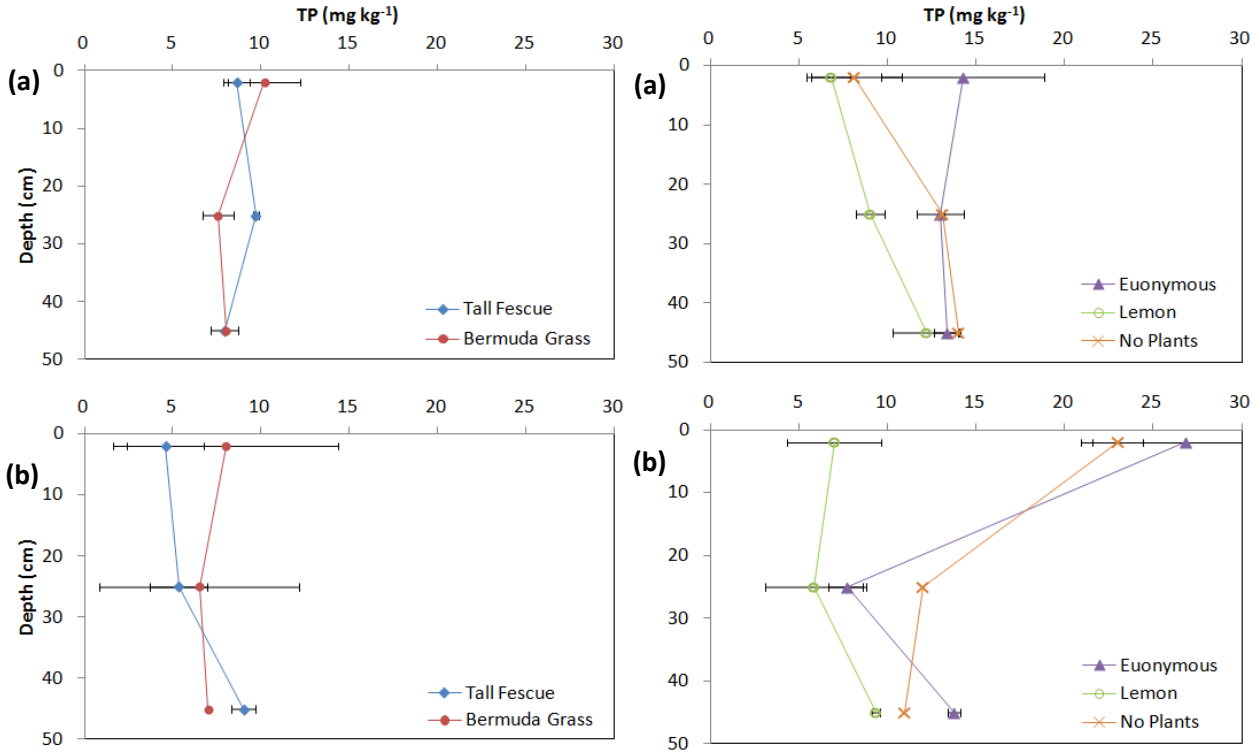

Figure 3-13. Total Phosphorus Measured in Soil Samples; (a) Potable Water-Irrigated, (b) Graywater-Irrigated.

3.5.3 Organic Matter and Surfactants

Organic matter was measured soil samples (Figure 3-14). While surface soil samples collected from graywater-water irrigated columns had significantly higher organic matter (1.75±0.76 %; average over all planted and unplanted columns) compared to potable water-irrigated columns (1.42±0.40 %; P≤0.05), no significant difference was observed for organic matter values in depth soil samples (25 and 45 cm) collected from graywater and potable water-irrigated columns (P > 0.05; Figure 3-14).

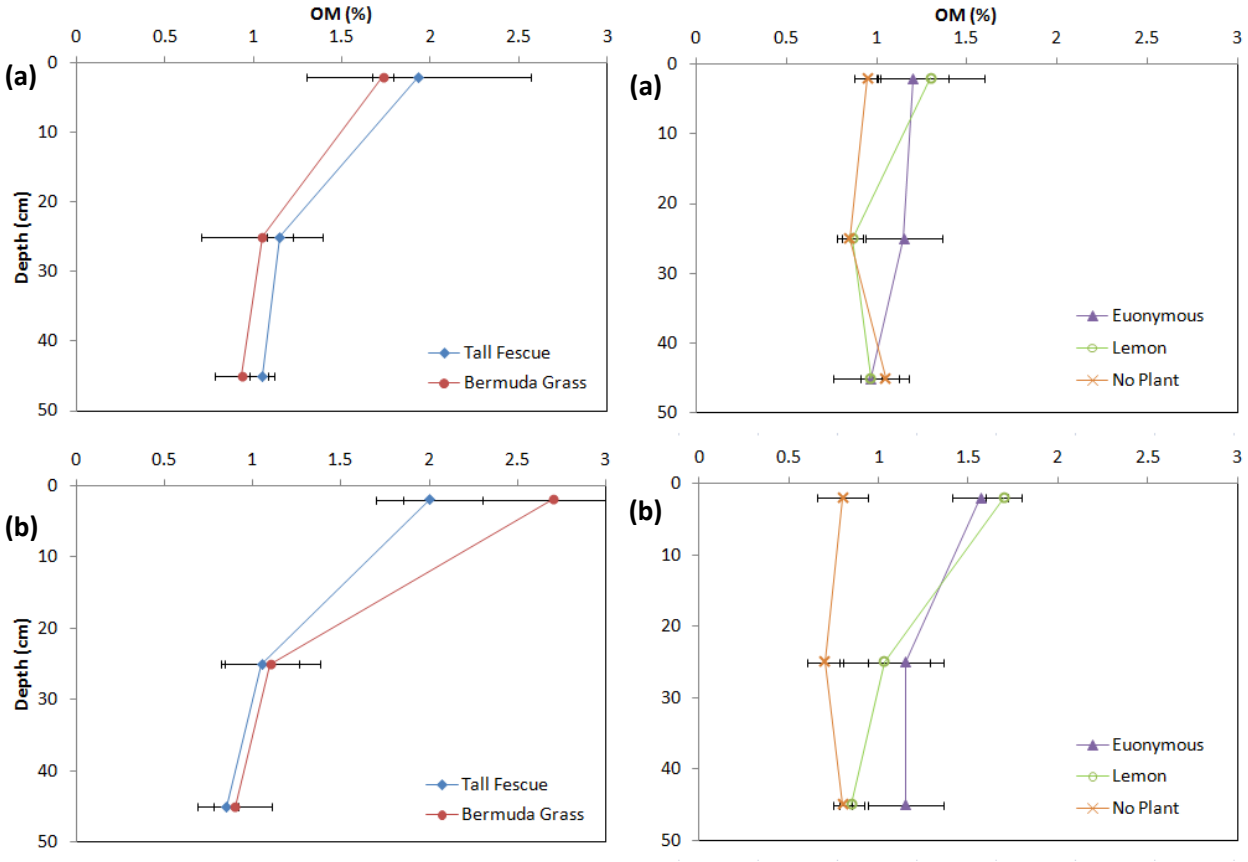

Figure 3-14. Organic Matter in Soil Samples; (a) Potable Water-Irrigated, (b) Graywater-Irrigated.

Total surfactants were determined in soil samples (Figure 3-15). Surface soil samples had significantly higher total surfactants than deeper soil samples (25 and 45 cm; Figure 3-15; P≤0.05). Total surfactant concentration in surface soil samples ranged from 940 to 2212 µmol kg^{-1}, while soil samples collected from 25 and 45 cm below the surface total surfactants concentration ranged from 44 to 117 and 3 to 28 µmol kg^{-1} respectively (Figure 3-15). This result indicated that surfactants accumulated in the surface soil samples due to potential adsorption to the soil particles, which prevented their transport to the deeper soil.

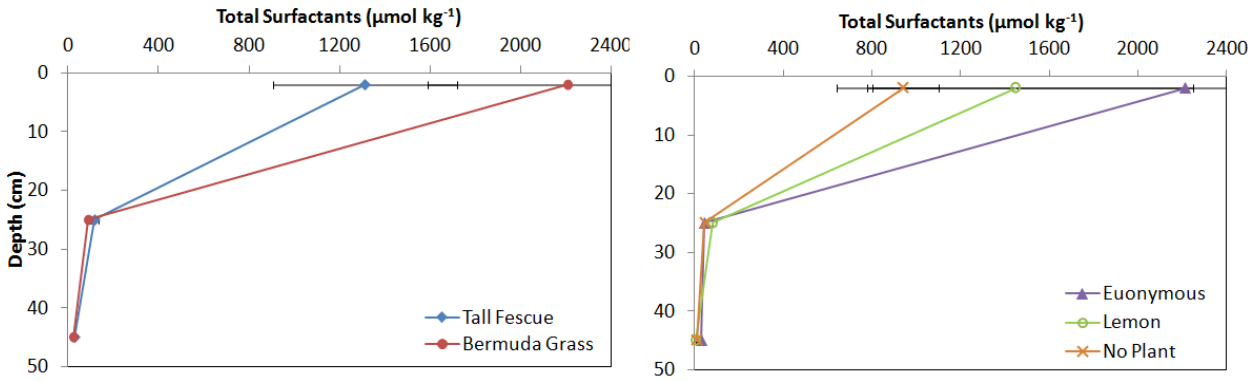

Figure 3-15. Total Surfactant Concentration in Soil Samples.

Surfactant concentrations measured in the greenhouse study were higher, but comparable to those reported in other studies. Travis et al., (2010) reported total surfactants 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, field samples collected from households in this study contained an average total surfactant concentration of 0.078±0.032 and 0.173±0.047 mg kg^{-1} for existing and new graywater installations respectively (Section 2.5.1.2 and Section 2.5.2.2). However, it should be considered that these studies were conducted under field conditions where soil received rainwater and graywater was not continuously applied for irrigation over the year.

A mass balance on surfactants was conducted using the concentration of surfactants in the soil samples, loading rate and leaching rate of surfactants. Simplifying assumptions were applied. The amount of surfactants accumulated in soil was determined for all measured surfactants including LAS, AES and AE separately. The average porosity of sandy loam used in the columns was assumed as 0.43. Results from surfactant mass balance 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 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 the 17 month duration of experiments.

The ratio of LAS:AES:AE was 78:16:6 in the synthetic graywater, and LAS was also the dominant surfactant in all of the soil samples (Figure 3-16). While higher LAS ratio was detected in surface soil samples, the relative content of AES increased with depth of soil samples (Figure 3-16). No AE was detected in 45 cm soil samples (Figure 3-16).

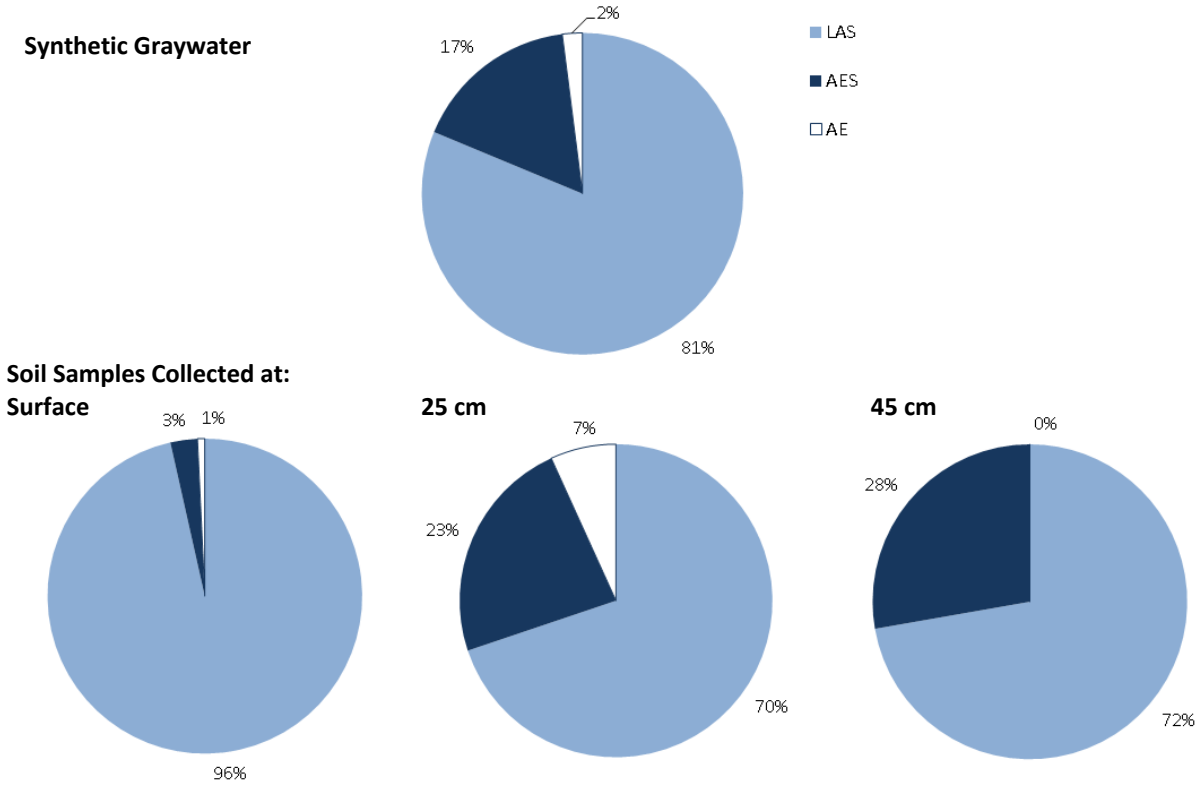

Figure 3-16. Surfactants Composition in Synthetic Graywater and Soil Samples. (calculated as molar mass)

3.5.4 Soil Infiltration Rate

A series of infiltration tests was conducted to investigate the effect of graywater application on soil structure. These tests were conducted on columns containing Euonumus, Lemon tree and unplanted columns. It was not possible to conduct tests in columns planted with grass without destroying the plant and root systems. Results showed that graywater planted columns had an average infiltration rate of 94.1±15.2 cm hr^{-1}, significantly higher than that measured in freshwater irrigated columns, 38.4±11.3 cm hr^{-1} (Figure 3-17). In the columns with no plant, graywater-irrigated columns had significantly higher infiltration rate than potable water-irrigated columns ($P<0.05$; Figure 3-17). This result showed that graywater application may have changed the soil properties in unplanted columns, resulting in increased infiltration rates. The same trend was observed at some of the testing events for columns with lemon (Figure 3-17). However, no significant difference was observed for infiltration rate in graywater and potable water-irrigated euonymus ($P>0.05$; Figure 3-17).

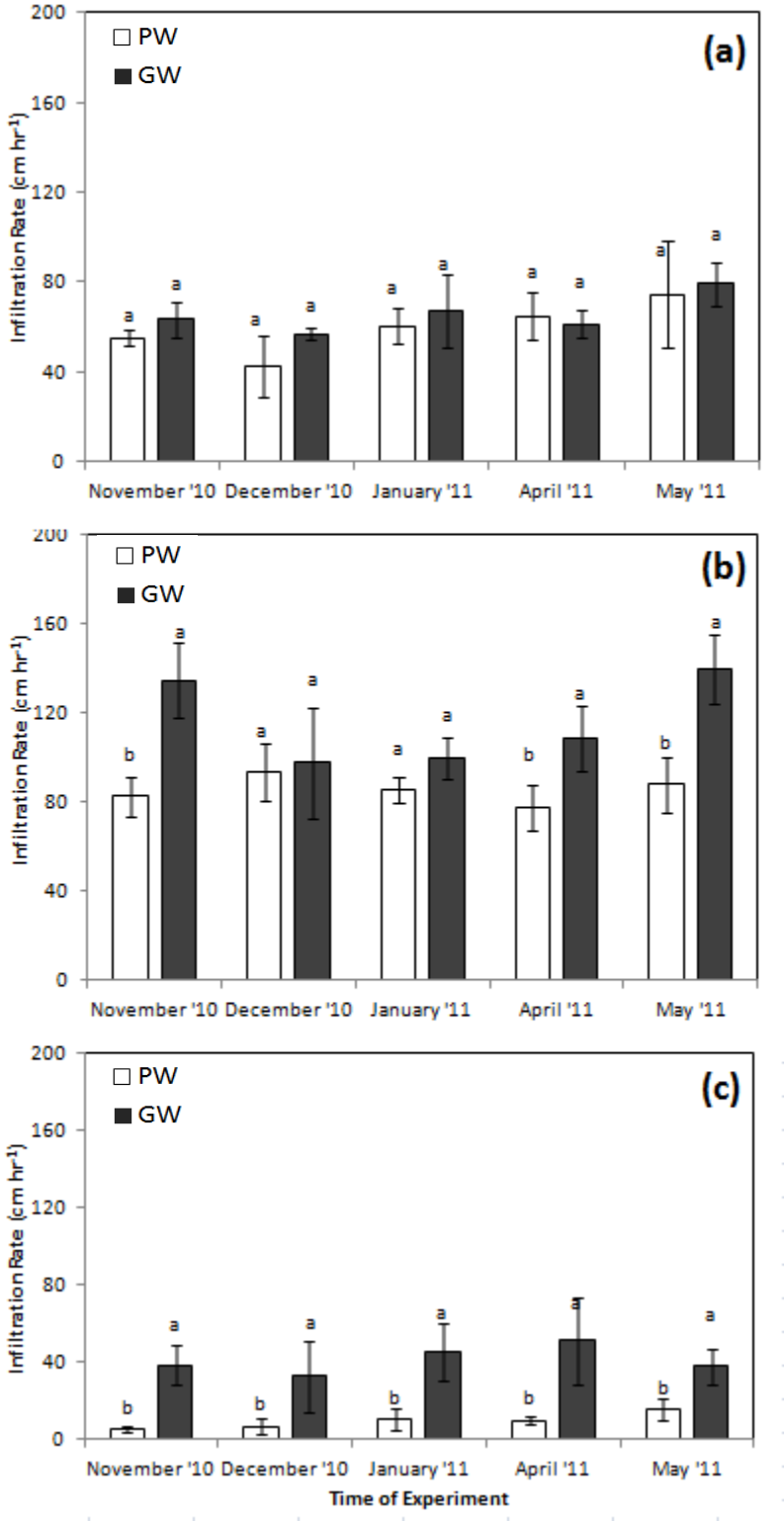

Figure 3-17. Infiltration Test Results: (a) Euonymus, (b) Lemon, (c) No Plant. (Nov and Dec: 2 replicates, Jan, April and May: 3 replicates; PW: potable water-irrigated, GW: graywater-irrigated; a, b: significantly different ($P \leq 0.05$); a, a: not significantly different ($P > 0.05$))

3.6 Plant Health

To evaluate plant health in the greenhouse study, the research team collected data on crown density, foliar color, turf quality, and above ground biomass for each treatment. Crown density for shrubs was rated on a 1-5 scale, where 5= optimum density. Foliage color of shrubs was rated on a 1-5 scale, where 5 = healthy color, and 1 = totally chlorotic. Turf quality was rated based on color, density, and uniformity using a scale of 0 (brown, dead turf) to 9 (optimum color, density, and uniformity), with a rating of 6.0 or higher indicating acceptable quality. At the termination of the experiment, total above ground biomass and above ground leaf biomass were collected. Biomass was oven-dried (at 70°C for 48 h) and weighed. At the termination of the experiment, leaf samples for all treatments were collected to test the mineral content; samples were analyzed for Cl, Na, K, Ca, Mg, B, and TN.

No visual symptoms of toxic effects were observed on any plant, regardless of the irrigation water type. Graywater-irrigated plants exhibited enhanced density, better color, and better quality when compared to potable water irrigated plants (Table 3-5). Both plant species and irrigation water source significantly affected aboveground biomass yield (Table 3-6). Graywater irrigation increased total aboveground biomass for all species. For the two turfgrass species, tall fescue and bermudagrass, the increase was 179% and 170%, respectively. For Lemon and Euonymous, the increase was 70% and 94%, respectively. Notably, graywater irrigation increased leaf biomass more than that of stems for the two shrub plants. The increase in stems was 56% and 65%, compared to 162% and 215% in leaves for lemon and Euonymous, respectively. This is due to the additional nutrients added from the synthetic graywater as indicated in the water and soil analyses. The total nitrogen content in graywater was 21 times greater than potable water (3.88 vs. 0.18 ppm) (Table 3-3). At the conclusion of the study, potable water irrigated columns had very low TN content, whereas graywater-irrigated surface soil samples had 2-4 times higher TN values than potable water-irrigated soil samples (Figure 3-12). Of note is that aside from the one time fertilizer application at the start of the study, no additional fertilizer was added to any of the treatments for the duration of the study (22 months). Both plant species and irrigation water source significantly affected plant mineral content in leaves (Figure 3-18). Sometimes the interactive effect of plant species and irrigation water source was significant. When compared with the potable water irrigated plants, graywater irrigation increased tissue B, Cl, and Na content in all plants with the exception that the research team did not observe Na increase in lemon plant (Figure 3-18). The degrees of tissue Cl and B increases under graywater irrigation were lesser for lemon than the other species (Figure 3-18). The relatively low Cl and Na in lemon when compared to other plants suggested that lemon may have excluded or sequestered these elements in their basal parts (roots or stems). However lemon had a higher level of B in the leaf tissue. Lemon is reported to be quite sensitive to B. In general, excessive accumulations of Na, Cl, and B would result in ion toxicity in plants, although the toxic thresholds likely differ for each element among different plant species.

The usual toxic ions in irrigation water are Cl, Na and B. Toxicity normally results when these ions are accumulated in the leaves during water uptake and transpiration to extents that damage to the plant. The degree of damage depends upon ion concentration and plant sensitivity. Damage can be caused individually or in combination. The low Na and Cl content in lemon leaves despite growth in Na and Cl rich conditions suggested that lemon may have low Na and Cl thresholds in plant tissue, i.e. it needs to maintain low tissue Na and Cl content to be healthy. Although grown in the Na and Cl rich environment in this study, lemon maintained lower levels

of Na and Cl, likely via mechanisms such as Na and Cl exclusion, regulation of transport to shoots, organismal Na and Cl compartmentations (Tester and Davenport, 2003).

For the beneficial elements, the research team observed a decreased Mg content in lemon and an increased leaf Mg accumulation in tall fescue, bermudagrass and Euonymous, in response to graywater irrigation (Figure 3-19). Graywater irrigation increased leaf Ca content in Euonymous and bermudagrass, while decreased Ca in lemon and tall fescue (Figure 3-19). When compared with the potable water irrigated plants, tissue K content increased in all plants (Figure 3-19). Tissue P content increased in the two grasses, but decreased in the two shrubs in response to graywater irrigation. Except for Euonymous where no change occurred, all species had increased tissue total N content under graywater irrigation; this was in agreement with the nitrogen deficiency appearance (yellow leaves and slow growth) observed for potable water irrigated plants(Figure 3-19).

Table 3-5. Crown Density and Foliar Color of Two Shrubs and Turf Quality of Two Turfgrasses Subjected to Two Different Irrigation Water.

Type of Plant	Species	Irrigation Water	
		Gray water	Potable water
		Crown density	
Shrub	Lemon	3.38 b*A**	2.50 bB
	Euonymous	4.69 aA	3.38 aB
		Foliar color	
Shrub	Lemon	3.31 bA	2.50 bB
	Euonymous	4.56 aA	3.50 aB
		Turf quality	
Grass	Bermuda	5.13 bA	3.63 bB
	Tall Fescue	6.25 aA	4.56 aB

*Lower case letter in the same column within the same plant type followed by different letters are significantly different at $P \leq 0.05$.
**Upper case letter in the same row followed by different letters are significantly different at $P \leq 0.05$.

In many plants, the reduction in tissue P, Ca, and Mg are associated with nutrient imbalance under saline environments. Despite the lesser increase of tissue Cl and B under graywater irrigation, it appears that lemon is the most affected plant by graywater irrigation in this study – it exhibited decreased Ca, P, and Mg content. This coincided with the fact that lemon showed the least growth increase stimulated by the N and P present in synthetic graywater. This is also consistent with field study results indicating sensitivity of lemon trees to graywater irrigation (Section 2.4).

Calcium may help to alleviate ion toxicity. The actions of Ca^{++} in salt stressed plants include the reduction of sodium binding to cell walls and plasma membrane, alleviating membrane leakiness, and preventing salt-induced decline in cell production and elongation, and improving uptake of important nutrients such as K^{+}. In a greenhouse study, Warren et al., (2004)

found that $CaCl_2$ amendment improved shoot growth and visual appearance of Petunia, Holly, and loblolly pine irrigated with untreated laundry graywater.

Table 3-6. Aboveground Biomass of Two Shrubs and Two Turfgrasses Subjected to Two Different Irrigation Waters.

Type of Plant	Species	Irrigation water	
		Gray water	Potable water
		Aboveground total biomass (g)	
Shrub	Lemon	80.7 b*A**	47.4 bB
	Euonymous	126.2 aA	65.1 aB
Grass	Tall fescue	117.0 aA	41.9 aB
	Bermudagrass	102.9 bA	38.1 bB
		Biomass of leaves (g)	
Shrub	Lemon	16.3 bA	6.2 bB
	Euonymous	39.4 aA	12.5 aB
		Biomass of stems (g)	
Shrub	Lemon	64.4 bA	41.3 bB
	Euonymous	86.9 aA	52.7 aB

*Lower case letter in the same column within the same plant type followed by different letters are significantly different at $P \leq 0.05$.
**Upper case letter in the same row followed by different letters are significantly different at $P \leq 0.05$

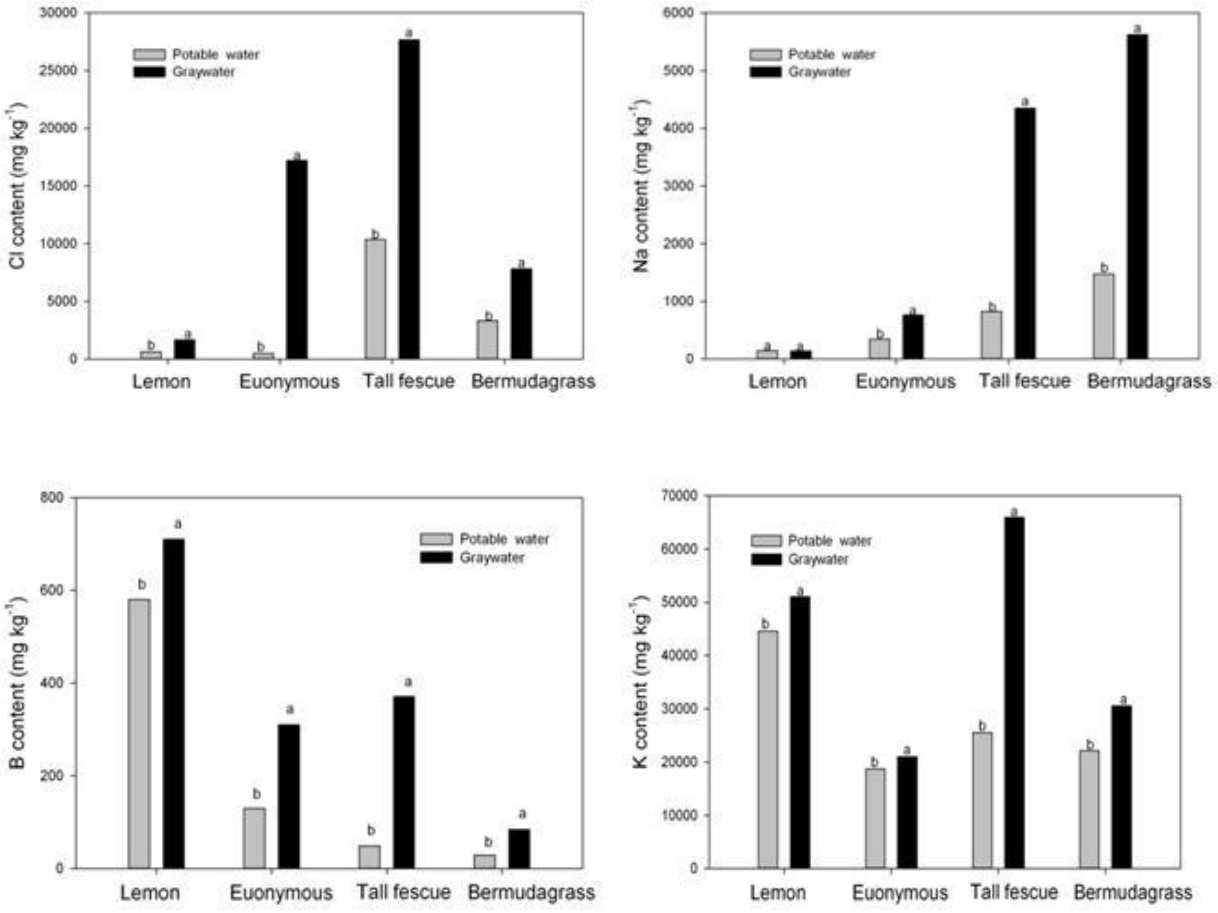

Figure 3-18. Leaf Na, Cl, B, and K Content of Two Shrubs and Two Turfgrasses Subjected to Two Different Irrigation Waters. (*Different letters for each mineral within the same plant indicate significant difference at $P \leq 0.05$)

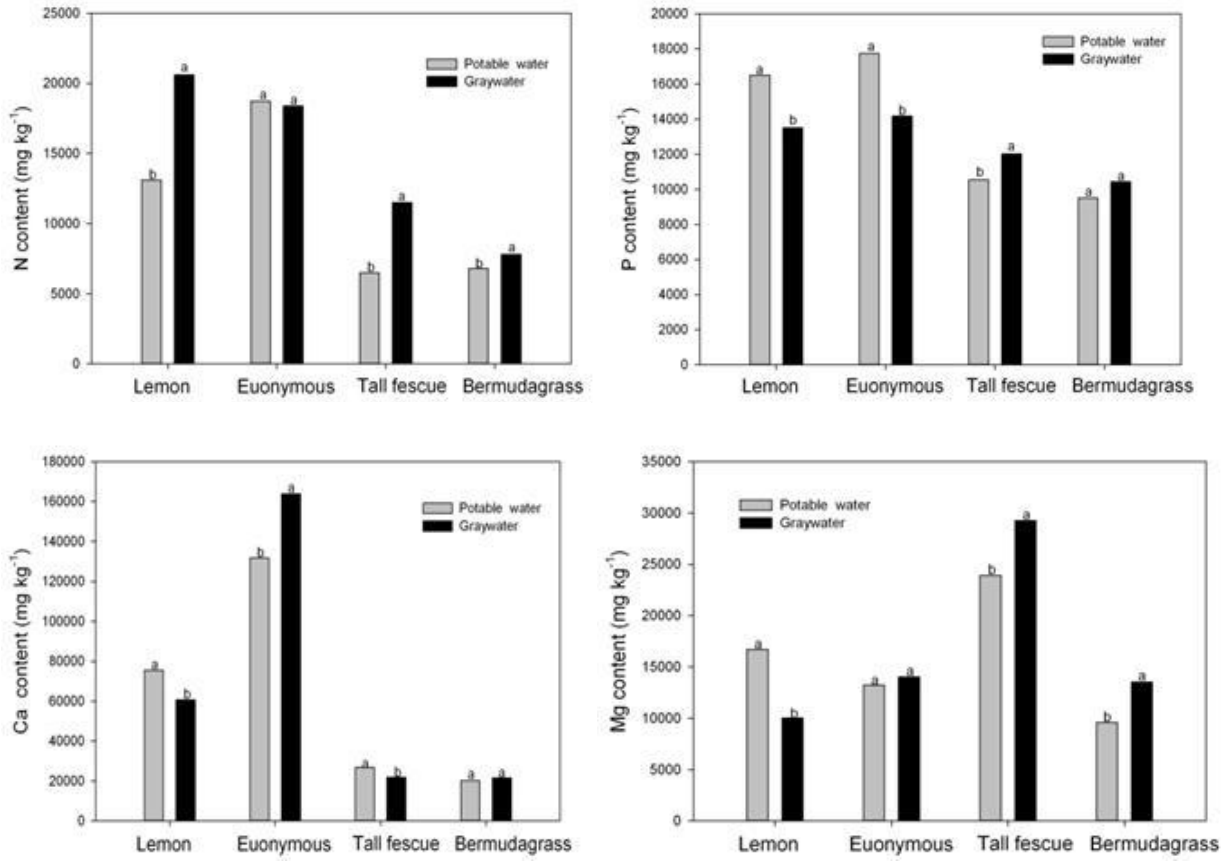

Figure 3-19. Leaf N, P, Ca, and Mg Content of Two Shrubs and Two Turfgrasses Subjected to Two Different Irrigation Waters. (*Different letters for each mineral within the same plant indicate significant difference at $P \leq 0.05$)

3.7 Summary

Graywater-irrigated columns generated less leachate than potable water-irrigated columns. Consistent with this result was a higher observed growth in graywater-irrigated plants than potable water-irrigated plants. Graywater-irrigated soil had higher TN values than potable water-irrigated soil, indicating additional nitrogen available in soils irrigated with graywater. While results showed accumulation of TN in surface soil irrigated with graywater, no accumulation was observed for TP in graywater-irrigated soil samples. No trend of TN and TP leaching was observed over the duration of the study and most of applied N and P were retained in the soil column.

TDS was higher in the leachate collected from graywater-irrigated columns than in the leachate collected from potable water-irrigated columns. Graywater-irrigated soil samples had higher SAR and EC values than potable water-irrigated samples. The SAR values remained, however, below 5, low enough to prevent any harmful effect for plants water uptake. Leachate and soil analysis showed a potential leaching of salts into the deeper soil as higher EC values were measured in graywater-irrigated soil samples than potable water-irrigated soil samples.

B was higher in the leachate collected from graywater-irrigated columns than in the leachate collected from potable water-irrigated columns. An increasing trend was observed for average B leached from graywater-irrigated columns. Despite the higher B content in graywater-irrigated soil samples than potable water-irrigated soil samples, B was still below the deteriorative level for plant growth of 5 mg kg^{-1} in all soil samples. More leaching was observed for B when grasses were present in the columns.

Less than 19% of added surfactants leached through columns. However, the amount of surfactant leached increased over the 17 month duration of experiment. Continuous irrigation with synthetic graywater resulted in accumulation of surfactants in surface soil samples. No accumulation of surfactants was observed in deeper soil samples. Graywater-irrigated surface soil samples had higher organic matter compared to freshwater irrigated soil, consistent with trends observed for surfactants. Concentrations of surfactant in surface soil at the end of this study ranged from 940 to 2212 µmol kg^{-1}, higher than observed in the field study (Section 2.5.3). Soil collected in the field was exposed to rainwater and this likely explains the higher concentrations observed in the greenhouse study where the only water received by graywater irrigated plants was synthetic graywater. Concentrations observed in the greenhouse study columns is representative of the high end of what would be observed in real world soil irrigated with graywater. Even at these concentrations, plant toxicity was not noted.

In conclusion, graywater irrigation represented beneficial effects on plant growth and added nitrogen to the soil. Results showed that soil-plant systems were capable of removing considerable amount of surfactants from the graywater. However, decreased surfactant retention over time raises concern over migration of surfactants to groundwater when graywater is applied for irrigation over a long duration. Results also raised concern over the leaching potential of salts, including N and B, into the deeper soil and possibly to groundwater. While SAR and B values were not accumulated in the soil samples above the harmful levels for plants health, further investigation are still required to evaluate the effect of graywater irrigation on soil quality.

CHAPTER 4.0
SUMMARY AND CONCLUSIONS

4.1 Graywater Effects on Plant Health

The research team found that most plants were healthy under long-term (more than 5 years) graywater irrigation. Among 22 plant species evaluated, the researchers only observed three species (Avocado, Lemon tree, and Scotch pine) that were sensitive to graywater irrigation, exhibited through reduced growth, or leaf burning, or reduced fruit production under graywater irrigation. Lemon trees also showed some early indications of toxicity in the greenhouse study. The research team did not observe consistent Na, Cl, and B accumulation in most evaluated species in the field. For the new installation household study, the most confident results were obtained from the AZ site. Graywater irrigation had positive impacts (higher shoot growth, better density, color, less degree of winter dormancy and overall quality) on bermudagrass, peach, and black-eyed Susan. Canna lily did not show differences between graywater and control treatments. Graywater irrigation had negative impacts on lemon and hybrid Rose, consistent with other results. For the CA and CO new household sites, other confounding environmental factors made the comparison of plants irrigated with freshwater and graywater inconclusive. Nevertheless, the research team observed no negative impacts on most of the evaluated landscape plants.

Due to the much greater nutrient content in the synthetic graywater for the greenhouse study, synthetic graywater-irrigated plants exhibited greater plant biomass and enhanced density, color, and quality when compared to potable water irrigated plants. No visual symptoms of toxic effects were observed.

4.2 Graywater Chemical Constituent Accumulation in Soil and Potential to Leach to Groundwater

Sodium accumulation has been a problem for reclaimed water irrigation and is also a concern for graywater irrigation. While SAR was sometimes larger in graywater-irrigated compared to freshwater-irrigated soil, SAR was always below 5 in soil samples, 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 housheolds 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 columns compared to potable water-irrigated columns. 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 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 the greenhouse study, TN leached from graywater irrigated columns as a percentage of mass added in graywater 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 uptaken by plants, there is still potential for nitrogen to leach through soil and to groundwater. Phosphorus did not accumulate in soil samples collected in the field study or the greenhouse study, and there was not a significant difference in phosphorus leached from graywater and potable water-irrigated columns.

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 receiving freshwater respectively. OM levels were not notably different at the CA (new installation) sampling location in the graywater-irrigated area compared to the freshwater-irrigated area. 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 also. An increase in OM is considered beneficial for both soil quality and plant health.

In field studies, graywater was determined to significantly impact surfactant concentration in soil. At all three households where a new graywater irrigation system was installed surfactants in soil did not increase over time. Instead, there was a notable increase from the baseline sampling event at the first sampling event after graywater irrigation was initiated and then surfactants 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 (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. In fact, toxicity was not observed in graywater irrigated plants in the greenhouse even when surfactant concentration was higher than observed in samples collected from households. 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. 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.

Even though antimicrobials were only detected in surface soil samples (0-15 cm) collected, 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.

For many of households studied in the field study, observed infiltration rates were higher in areas irrigated with graywater compared to freshwater. However, data was too variable to make strong conclusions on the impact of graywater irrigation on infiltration. Infiltration tests were also conducted on planted columns in the greenhouse study. Here, infiltration rates were always higher in graywater irrigated columns and the difference was statistically significant in unplanted columns ($P \leq 0.05$). Based on combined results from the field and greenhouse studies, it can be concluded that long-term graywater irrigation may increase soil infiltration rate.

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.

To summarize the potential for graywater constituents to leach into groundwater, 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 uptaken 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.

4. 3 Graywater Effects on Fecal Indicator Bacteria in Soil

Graywater has the potential to contaminate the environment with human-associated fecal organisms, including *E. coli* and enterococci. In this study, however, the research team found no strong, consistent effect of graywater on estimates of *E. coli* or enterococci in soil. Contamination was inconsistent and depended on the household, sampling date, and depth of soil sampled. In addition, *E. coli* and enterococci were detected in freshwater-irrigated soils, indicating sources other than graywater for fecal indicators detected in the environment. Quantitative microbial risk assessment may be another way to evaluate risk associated with pathogens resulting from graywater irrigation, such as the work conducted by Maimon et al. (2010).

4.4 Recommendations for Graywater Irrigation

No major concerns were identified in this study that would render reuse of graywater following best management practices unsafe for human activities. The state of Arizona has set the standard for graywater irrigation best management practices (http://www.azdeq.gov/environ/water/permits/download/graybro.pdf) and these practices are recommended in many states. Graywater does contain pathogens and human contact with graywater should be avoided. Graywater should be applied through drip irrigation with a protective layer of mulch above emitters. In some states, submerged irrigation systems are

required. One such system was studied as part of this research. There was no indication that a submerged irrigation system resulted in lower indicator organisms compared to surface irrigation systems studied here. In general, the source of indicator organisms was difficult to determine since they were found in areas irrigated with freshwater. However, because indicator organisms were detected in graywater irrigated areas, it is recommended that human contact with graywater irrigated areas is avoided. Placing a mulch layer over drip emitters where graywater is applied is good measure to minimize human contact with graywater irrigated soil. The research team found that most plants are healthy under long-term graywater irrigation. However, Avocado, Lemon tree, and Scotch pine are sensitive to graywater irrigation and not recommended when graywater is the only source of irrigation water. Results from the greenhouse study showed that N present in graywater was beneficial for plant growth. In the case that one decides to apply graywater for irrigation, fertilizer will be needed in lower quantity than potable water irrigated areas, if needed at all. TN in graywater can range from 19-80 mg L^{-1} and one can estimate the amount of N added through graywater to determine how much additional fertilizer may be required. Because B and antimicrobials were determined to potentially accumulate in soil irrigated with graywater, products containing these ingredients are not recommended when graywater will be applied for irrigation.

4.5 Recommended Future Work

While this project was the most comprehensive study to date on impacts of graywater reuse for irrigation, 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 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. 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. Further research is required to assess risk associated with pathogens and viruses in graywater.

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APPENDIX A

QAPP

QAPP for the Research Project:

A Long-term Study on Landscape Irrigation Using Household Graywater – Experimental Study

June 5, 2008

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Principal Investigator: L.A. Roesner, Professor, Colorado State University

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CSU Quality Assurance Manager: Mary Stromberger, Associate Professor

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WERF Project Manager: Jeff Moeller

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WERF Quality Assurance Manager:

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WERF Director of Grants, Audits, and Procurement Programs:

A2: Table of Contents

Group A: Project Management

A3: Project Organization and Schedule

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Participant Responsibilities

CSU has assembled a strong team that includes a blend of expertise in both practical engineering problems and science. Three of the principal team members have direct experience with household graywater reuse, and/or reuse of wastewater treatment plant effluent for landscape

irrigation. In addition, three of the principal team members were involved with Phase I of this project, Literature Review and Draft Experimental Plan. Dr. Larry Roesner will serve as Principal Investigator. Dr. Roesner has expertise in Water Quality Engineering and will lend his expertise in graywater system design and reuse. Dr. Sybil Sharvelle will be Co-PI and Project Manager; she will be the principal point of contact with the WERF Project Manager. Dr. Sharvelle has experience with studying the fate of surfactants and also will be responsible for measurement of surfactants and antimicrobials in graywater and soil. Dr. Mary Stromberger is a soil scientist and will be responsible for soil chemistry as well as soil microbiology and pathogen studies. Dr. Yaling Qian is a horticulturist and will be responsible for overseeing the horticultural aspects of the project.

Project Schedule

The timeline shown in Figure 1 outlines the proposed 3 year timeline for this project, including expected times for graywater, soil, and plant sampling. Included in the timeline are experiments on households with existing graywater irrigation systems (Part 1), experiments on households installing new graywater irrigation systems (Part 2), and greenhouse studies (Part 3).

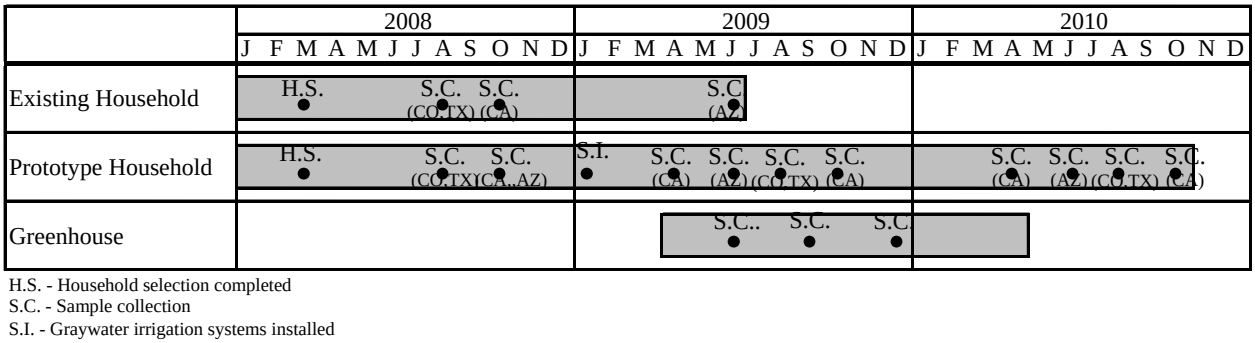

Figure 1. Timeline for completion of experimental work.

A4: Problem Definition and Background

As communities throughout the United State and abroad are becoming interested in innovative approaches to water resource sustainability, household graywater reuse for residential landscape irrigation is gaining popularity. In a typical household, graywater (near 28 gallons per person per day) is nearly 50% of the total wastewater generated. 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. 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%). Some states, including California, Arizona, and New Mexico have legalized the practice.

There are potential risks associated with graywater reuse for irrigation. The physical, chemical, and microbial characteristics of graywater are highly variable based upon the sources connected to the collection system, household inhabitants, household chemicals used by the residents for personal hygiene and house cleaning, personal care, plus medications and waste products disposed of in sinks (Eriksson et. al., 2002). Application of graywater may result in increased levels of pathogens and viruses, negative impacts to soil quality, potential groundwater contamination with chemical constituents present in graywater, or negative impacts to plant health. These risks should be further evaluated and methods of graywater application that minimize these risks should be understood.

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. However, the fate of pathogens after graywater application is not well understood and their persistence could result in human health risks.

In addition, application of graywater for irrigation may impact soil chemistry. Potential effects of graywater on soil chemistry include changes in pH, salinity, and concentrations of chemicals, specifically organics and metals, introduced by the graywater. Very few published studies were found that evaluated these changes in the soil. The Gray Water Pilot Project in the City of Los Angeles, CA (1992) conducted research on eight voluntary residential sites retro-fitted with graywater systems for the purpose of residential sub-surface irrigation. Results showed an increase in sodium levels (Na+) and in the Sodium Adsorption Ratio (SAR).

Changes in soil chemistry may also 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 effect of graywater irrigation on plant health.

While graywater reuse for household irrigation is widespread, potential effects on soil quality, groundwater quality, and plant health 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. 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. Current research has not 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 study proposed herein describes scientific experiments to alleviate these information gaps regarding household graywater irrigation.

Objective

The objective of this research project is to elucidate information on the fate and occurrence of graywater chemical constituents and pathogens and their potential impacts on soil quality, groundwater quality, and plant and human health as a result of its application for residential landscape irrigation. Field and greenhouse studies will be the focus of our research efforts so that data collected can be directly used by regulatory agencies and home owners interested in graywater irrigation application. Households in different climatic regions will be selected so that recommendations relevant to these climatic regions can be made. Quantitative data collected on the fate of graywater constituents and effects on plant health will provide a factual based framework for decision making regarding safe reuse of graywater for residential landscape irrigation.

A5: Project Description

The project description is detailed in full in the proposal. In brief, a three part study with a duration of three years has been proposed. First, soil samples will be collected at several household sites that have been using graywater for irrigation for more than 5 years and compared with analogous soil and landscaping that has been irrigated with potable water. It is expected that the operating protocols for these systems will not be well documented, so in the second and third parts of the study, controlled graywater application experiments will be conducted to achieve more scientifically defensible data. The second part of the study will target new applications of graywater to several selected sites, in different climatic regions. These sites will be operated in a controlled manner for 2 years to determine the dynamics of changes to soil and plant health that might occur due to graywater irrigation and the risk to human health in new systems. During these field experiments, graywater samples, soil samples, and plant samples will be collected to determine the effects of graywater constituents and pathogens. In addition to the field studies, a greenhouse experiment will be conducted at no cost to WERF to evaluate toxicity of graywater to plants and to monitor leachate from graywater irrigated soils.

The research proposed herein will provide scientific data on the fate and occurrence of graywater chemical constituents and pathogens so that potential impacts to soil quality, groundwater quality, and plant and human health may be evaluated. Quantitative data collected during these experiments will provide guidance to decision makers, water agencies, regulators, product manufactures, and consumers so that safe graywater irrigation systems can be installed and operated for household irrigation. This study will alleviate existing knowledge gaps that have prevented widespread reuse of graywater for irrigation.

A6: Quality Objectives and Criteria for Measurement Data

The Quality Assurance objectives are to:

1. Assess each step in the overall system so that the analysis is consistent with each batch of samples analyzed.
2. Evaluate instrument performance and assess maintenance requirements.

3. Improve the field and lab techniques so that methods are performed according to Standard Operating Procedures (SOPs).

4. Identify non-routine samples that may not comply with the normal QA/QC procedures and may require special attention or a modification in the SOP.

Regularly scheduled meetings among project managers and participants will monitor project performance. Project performance also will be judged based on implementation and completion of project tasks according to the schedule on page 5.

Soil and Plant testing laboratory performance criteria and analytical instrumentation tolerable limits are addressed in the Appendix. Briefly, reagent blanks will be used with each sampling batch, along with ten percent duplicates per batch. An in-house soil and plant standard will also be utilized, both of which have been analyzed > 25x to assure quality. ICP-AES and Alpkem and LECO-1000 tolerable limits = 10%, pH meter must be within +/- 0.05 units during standard analysis, EC meter will be calibrated with 0.01M KCl solution.

For statistical analysis, the research team will conduct analysis of variance tests followed by least significant difference procedure to separate treatment means, using a probability level of both 90% and 95%. The experimental design for the existing household study is a split plot block design, with household locations serving as blocks (n=4), graywater versus potable water as main treatments (n=2) and sampling depth as the split effect (n=3). The experiment design for the new household/prototype study is a repeated measures split plot block design because samples are collected over time. The experimental design for the greenhouse study is a complete randomized two-way factorial design, with plant species (n=4) as one factor and water treatment (graywater vs. potable water) as the second factor. All univariate analyses will be performed using SAS statistical software (SAS Institute Inc., Cary, NC). Multivariate analysis of microbial community structure will be performed using the PC-ORD statistical software (MjM Software, Gleneden Beach, OR).

A7: Special Training Requirement/Certification

Not applicable.

A8: Documentation and Records

Homeowners will be required to maintain a log of all household products used that enter into the graywater irrigation system. In addition, the owners of households with newly installed graywater systems will be required to measure the volume of graywater produced on a daily or

weekly basis and alert Colorado State University researchers if landscape appears to be unhealthy.

The most current QA Project Plan, SOPs, and other documents will be distributed to the other investigators via email immediately after changes are made. A brief description of the QA modifications will also be attached to the email.

Field and greenhouse notebooks will be kept for documenting sampling events, including soil, water and plant sample collections, notes on plant visual inspections, and other relevant information. Laboratory notebooks will be used to document water quality, soils, microbial, and plant data. The data will be transferred to MS Excel and saved on both a hard drive and a CD-RW. Test method raw data and QC sample records will be saved directly to disk, a hard drive, and a CD-RW disk.

SAS model input and output files will be documented and saved along with all collected analytical data.

Group B: Data Generation and Acquisition

B1: Experimental Design

Sampling Frequency

Households from the Existing Household study will be sampled one time, while samples will be collected each year of the three year project for households with installation of new graywater irrigation systems. The 2008 sampling year of new households will generate background data, prior to onset of graywater irrigation. The 2009 and 2010 sampling years will generate data on one and two-year effects of graywater irrigation on plants and soils. The timing of sampling for locations of selected households has been determined based on average monthly precipitation (Figures 2-5) and the growing season. For CA, early October and late April have been determined to be optimal sampling times. This timing provides sampling at both the beginning and end of the rainy season (Figure 2). Samples from the existing household (Escondido) will be collected once in Ocotober of 2008, prior to the rainy season. Both locations in AZ (Bisbee and Tucson) receive most rainfall between July and March (Figure 3). Therefore, samples will be collected in June in AZ, near the end of the dry season. Because June of 2008 has already passed, the research team will collect existing household samples in June of 2009. Baseline samples will be collected at the new installation household in Phoenix in October of 2008. For all sites in CO and TX, samples will be collected in August or September, near the end of the dry season (Figures 4-5) and growing season.

During the greenhouse study, leachate samples will be collected three times during the study. Plant tissue samples and soil samples will be collected at the termination of the study.

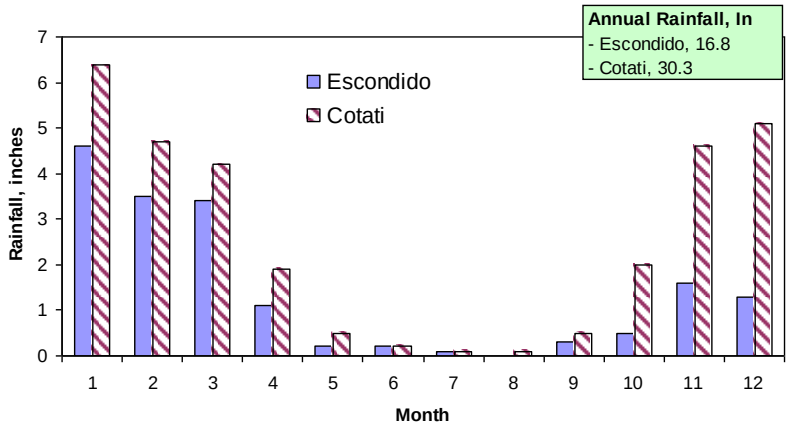

Figure 2. Average monthly precipitation for Sites Selected in CA.

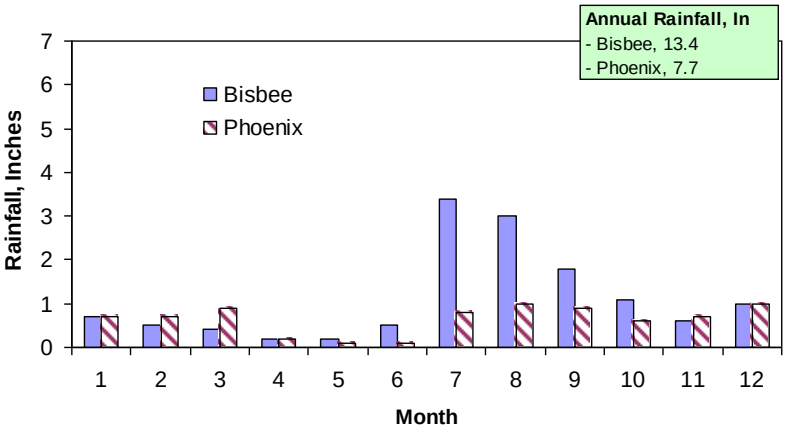

Figure 3. Average monthly precipitation for Sites Selected in AZ.

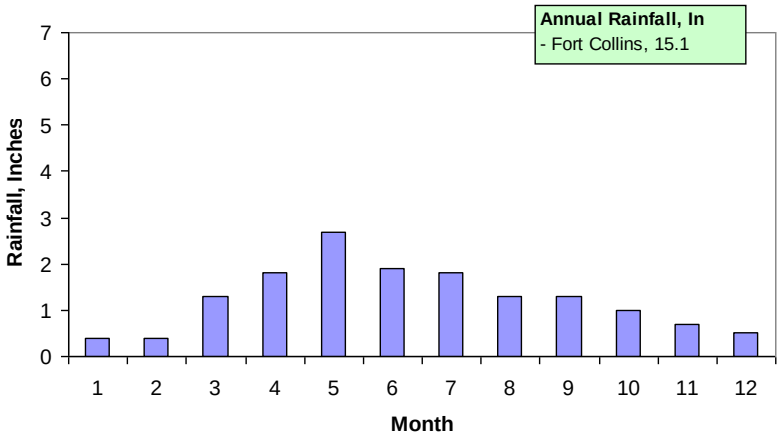

Figure 4. Average monthly precipitation for Sites Selected in CO.

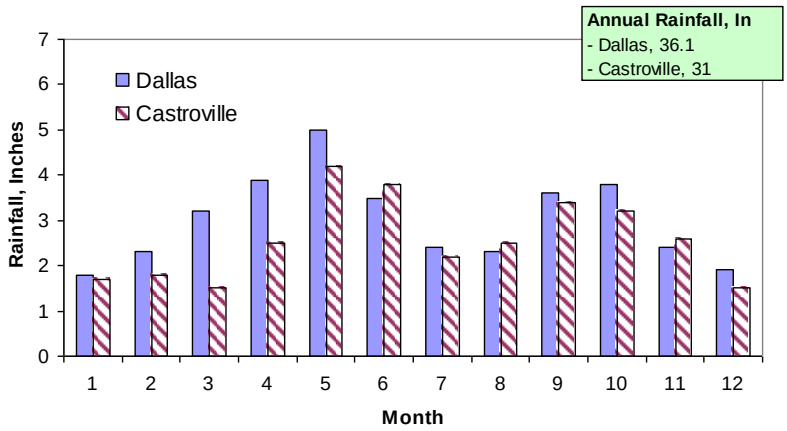

Figure 5. Average monthly precipitation for Sites Selected in TX.

Sampling Strategy (Household Studies)

The research team will coordinate with household owners to schedule sampling dates and times. At each household, plant and soil samples will be collected from a landscape area irrigated with graywater (treatment area) and an area irrigated with potable water (control area). Prior to sampling, household owners will have utility companies locate all buried electric, water and phone lines so that the soil sampling is not conducted over buried lines. Owners of newly installed graywater system will provide a fresh graywater sample at the time of soil and water sampling.

B2: Sampling Methods Requirement

SOP for Graywater Sampling (Existing Households)

Graywater samples will be collected from plumbing post storage/treatment by a valve. The sample location will ensure that collected graywater samples are representative of water applied to irrigation. In the case that sites are included where a storage tank is not in place and graywater is applied as generated, a graywater sampling technique will be employed to ensure that collected samples are representative of average graywater composition rather than instantaneous composition. For example, the research team will request that the homeowner allow the graywater to drain into a 30-40 gallon basin for one day prior to sampling, and water samples will be collected from this basin. Graywater samples will be collected in 2L aliquots and stored in plastic bottles labeled with the household study site location and date. Graywater samples will immediately be placed in coolers with "blue ice" packs. Due to the ubiquitous use of surfactants, an extensive cleaning procedure will be applied to these bottles. Prior to being sent to the field, bottles will be rinsed with distilled deionized (DDI) water, 1:1 hydrochloric acid/water, and three rinses with high purity methanol. Samples will be preserved with 8% (v/v) formalin upon collection.

SOP for Soil Sampling (New Installation and Existing Household Studies)

Soils will be sampled with the same protocols across all study sites. Soil will be sampled adjacent to plants that have received gray- or potable irrigation water, with three soil cores taken per treated and per control area. Each soil core will be collected with a Zero Contamination sampling tube (0.8 inch diameter) connected to a Backsaver Handle (JMC Soil Samplers, Newton, IA). The sampling tube will be lined with a removable PETG copolyester liner to encase the sample and prevent contamination with surrounding soil as the soil sample is pulled up to the surface. Soil cores will be collected to three depth increments: 0-15 cm, 15-30 cm, and 30-100 cm. After sample collection, the removable liner will be sealed with vinyl caps, and the liner will be with the household study site location, treatment, soil depth, and date. All liner-encased soil cores from the same household, treatment and sampling depth will be placed into a large, labeled Ziploc bag and placed on ice in a cooler. Soil bulk density will be calculated based on the volume of sampling depth contained within the liner, the mass of the field-moist core, and the gravimetric moisture content of the soil core, determined on a subsample. All holes left in the yard from soil sampling will be filled with topsoil, purchased at a local home improvement store.

Single ring infiltrometer tests will be applied to each household site to estimate the infiltration capacity of the soils. Mulch or ground cover will be removed prior to initiation of tests. The infiltration tests proposed will be simple, consisting of a piece of 12 inch corrugated pipe placed on end and rotated with vertical pressure until it penetrates the exposed soil (i.e., after brushing aside any much) to a depth of 1 ½ to 2 inches. The pipe will then be filled with six to eight inches of water, and the rate at which the water surface falls will be measured. The test will be performed at a minimum of three locations per treated or control area. This provides a good estimation of surface soil infiltration rate; it is commonly used for estimating infiltration rate on soils proposed for use as stormwater infiltration facilities.

SOP for Plant Biomass Sampling (New Installation and Existing Household Studies)

Plant types to be examined and sampled include trees, shrubs, bedding plants, and turfgrasses. Landscape plants will be evaluated for their health and growth as follows:

Trees: Trees are to be evaluated in late summer; after the flush of growth has matured, for:
1) Health: Tree health will be assessed by developing health indexes based on the following criteria: crown density (1-5 scale), dieback from tip (absent or present), foliage color (chlorosis) (1-5 scale), suckers or water sprouts (absent or present), presence of insects, disease, and gummosis (absent or present), number of years of needle retention (factor of stress and genetics for evergreens).
2) Growth: For woody plants, historical growth data will be examined and measured by evaluating bud scales and internodal lengths for 4 representative branches per tree. These evaluations will provide information on year-to-year growth variations and the trend of the growth rate changes over time.
3) The percent foliar burn (leaf scorch and necrosis) will be estimated visually.

4) Leaf size: Average leaf size will be determined by measuring with LI-COR 3100 leaf area meter.

Shrubs: Shrubs may require pruning; instructions for pruning will be provided to the homeowners. Shrubs are to be evaluated in summer for:
1) Health: Shrubs will be assessed by developing health indexes based on the following criteria: crown density, shoot dieback, foliage color (chlorosis), presence of insects or disease, and number of years of needle retention for needled evergreens.
2) Growth: The current year's growth will be measured on four representative stems.
3) The percent foliar burn (leaf scorch and necrosis) will be estimated visually.
4) Leaf size: Average leaf size will be determined by a LI-COR 3100 leaf area meter.

Bedding plants: Bedding plants will be planted for each experiment year. Within each household pair (graywater irrigated vs. potable water irrigated), bedding plants can be planted at the same time with the same species and cultivars. Bedding plants are to be evaluated in early to late summer for:
1) Health: Bedding plant health will be assessed based on the following criteria: crown density, dieback from tip, foliage color (chlorosis), and presence of insects or disease.
2) Growth (vigor): plant size (height and diameter) will be measured. Bloom will be measured by counting the number of blooms on representative plants and estimating overall percent bloom.
3) The percent foliar burn (leaf scorch and necrosis) will be estimated visually.
4) Leaf size: Average leaf size will be determined by a LI-COR 3100 leaf area meter.

Turfgrass: During evaluation years, turfgrass maintenance, including mowing frequency, fertilization, aeration, and weed control will be standardized for each pair of the landscapes. Turfgrass will be evaluated as follows:
1) Turf Quality: turfgrass quality will be assessed based on canopy color, shoot density, uniformity, presence of weeds, disease, or insects. Turf quality will be evaluated based on a 1 to 9 scale.
2) The percent leaf tip necrosis will be estimated visually before mowing events

Landscape sample collection: Plant sampling protocols will consider the growth habit and typical management of each type of landscape plants. For conifer trees, the research team will collect about 50 grams (a handful or half of a small paper lunch bag) of 2 year-old foliage from at least 3 different branches. The research team will collect half a paper lunch bag full of leaves from deciduous trees in the same manner, and the research team will avoid very mature leaves or not fully developed leaves. The research team will also avoid collecting needles or leaves from shaded or the lowest branches. For bedding plants, the research team will randomly sample 10 fully developed leaves for tissue analysis. For turf, the research team will remove all vertical foliage above the thatch layer at a representative location to fill half a paper lunch bag. All bags will be labeled with the household study site location, plant type, treatment, and date. Once bagged, samples will be kept out of direct sunlight. At least one photo of each plant sampled will be taken with a digital camera to assist with plant identification later on.

<u>SOP for Plant Biomass Sampling (Greenhouse Study)</u>

At the termination of the greenhouse study, leaves from bedding plants and vertical foliage from turf will be collected as descibed above.

SOP for Leachate and Soil Sampling (Greenhouse Study)

Leachate and soil samples from greenhouse containers will be collected three times during the one year duration of the study in three of the four replicate PVC pots (6 inch diameter and 18 inches deep) for each of the four plants included. Included in this study will be two common bedding plants, Imapatiens and Geranium, and two common turfgrasses, Bermudagrass and Bluegrass. These plants will be planted in a total of 38 pots. Each pot will be packed with the same mass of soil to the same bulk density. Leachate samples will also be collected from one of the four replicate pots for each plant being treated with potable water to serve as a control.

B3: Sample Handling and Custody Requirement

Graywater Samples

On the same day as sampling, graywater samples will be transported by airplane (if project personnel return to CSU the same day) or shipped overnight (if project personnel do not return to CSU the same day) to Colorado State University in sealed coolers containing ice packs. Samples collected in Colorado will be transported by CSU personnel authorized by the principal investigators. Samples will be transported in their original containers. Upon arrival at CSU, 100 mL of the sample will be allocated into a sterile bottle for analysis of indicator organisms. In addition, 750 mL of sample will be allocated for analysis of TSS and TDS. The remaining graywater will be filtered through 0.2 μm cellulose acetate filters to remove microorganisms. The filtered sample will be stored at 4°C and utilized for chemical analysis within 14 days.

Soil Samples

The remaining soil samples will be transported the same day by airplane (if project personnel return to CSU the same day) or shipped overnight (if project personnel do not return to CSU the same day) to Colorado State University in sealed coolers containing ice packs. Samples collected in Colorado will be transported by CSU personnel authorized by the principal investigators. Samples will be transported in their original containers. Immediately upon arrival to Dr. Stromberger's lab, soil cores per each household location × treatment × sampling depth combination will be weighed, mixed together and then homogenized by hand. A subsample of each soil will immediately be analyzed for total coliforms, *E. coli*, and enterococci in Dr. Stromberger's lab. Another subsample of each soil will be collected for surfactant and antimicrobial analysis and delivered to Dr. Sharvelle's lab; this sample will be stored in a freezer when extraction can not take place immediately upon arrival.

A third subsample from soils of the existing household study will be shipped overnight to EMLab Pand K Laboratories in San Bruno, CA for most probably number (MPN) enumeration

of *Clostridium perfringens*. As stated in the proposal, soils and graywater from the new household/prototype study will not be analyzed for *Clostridium perfringens* because of budget constraints. The research team chose to analyze soils from the existing household studies only for this particular pathogen, because these soils will have the longest history in receiving graywater, with more time for this pathogen to accumulate to detectable numbers.

The remaining soil will be passed through a 2-mm sieve to remove roots and coarse fragments. The sieved soil will then be subdivided into three portions and stored in zip-lock freezer bags under conditions appropriate to a given analysis. Soil subsamples will be immediately analyzed for gravimetric water content. Subsamples for dehydrogenase enzyme activity will be stored at 4ºC prior to analyses, which will begin within 2 days of sampling. Soil subsamples for physical and chemical analysis will be air-dried and stored at room temperature prior to the analyses. All soil samples will be preserved at their respective storage facilities until all data has been collected, reviewed, statistically analyzed, and reported.

Plant Samples

On the same day as soil sampling, plant samples in paper bags will be packed into a cardboard box and will be transported by airplane as luggage (if project personnel return to CSU the same day) or shipped overnight (if project personnel do not return to CSU the same day) to Colorado State University. If plant tissue is succulent, plant tissue will be packed in a cooler with ice packs for deliver. Samples collected in Colorado will be transported by CSU personnel authorized by the principal investigators. Samples will be transported in their original containers. Shipping: If possible, ship samples the same day. Immediately after sample arrives in the lab, lightly rinse foliage with distilled water prior to oven dry the samples to eliminate dust or aerially deposited salts.

Greenhouse Study Leachate and Plant Samples

Leachate and greenhouse soil samples will be placed in a cooler on ice and walked over to Dr. Sharvelle's laboratory immediately after collection. Plant samples will be placed in paper bags and walked over to Dr. Qian's laboratory after collection.

B4: Analytical Methods Requirements

Graywater Samples

Graywater samples will be analyzed for standard water and wastewater parameters by methods as outlined in Greenberg et al., (1992). Analysis will include biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), TSS, TDS, pH, oxidation reduction potential (ORP), conductivity, total nitrogen (TN), ammonia (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), total phosphorus (TP), PO_4, and hardness. The specific method number and description for each of these analyses are provided in Table 1. Trace metals (iron, zinc, copper, chromium, nickel, cobalt, vanadium, molybdenum, and selenium) and boron will be measured by ICP (Table 1). These analytes will be measured by the CSU Soil, Water and Plant Testing Laboratory. Surfactants including soap, linear alkyl benzene sulphonates (LAS),

alcohol ethoxylates (AE), and alkyl ethoxy sulphates (AES) will be quantified by liquid chromatography mass spectrometry (LC-MS) as detailed elsewhere (Dyer et al.,, 2006; Sanderson et al.,, 2006a; Sanderson et al.,, 2006b). The LC-MS instruments to be utilized are in the CSU Central Instrument Facility. Surfactants are expected to be present in graywater samples above 1 mg/L and therefore a concentration step will not be required to achieve the desired detection limit. Antimicrobial additives commonly present in personal care products, triclosan (TCS) and triclocarban (TCC) will also be monitored using an LC-MS by methods as detailed by Halden and Paull (2005). Prior to analysis samples will be passed through a solid-phase extraction (SPE) cartridge (Oasis HLB, 3 cm3/60 mg sorbent; Waters Corp., Milford, MA). Eluates were dried under a gentle stream of nitrogen, reconstituted (1 mL, 50:50 methanol:acetone), filtered (0.2 μm), and reduced to initial eluent strength by dilution with water if needed.

Graywater will also be analyzed immediately analyzed in Dr. Stromberger's laboratory 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. Ten-fold serial dilutions of graywater will be prepared in sterile glass bottles, using sterile distilled water as the diluent. To prepare the dilutions, 22.2 mL of graywater will be diluted in 199.8 mL of sterile distilled water to create a 1:10 dilution. This dilution will be vortexed for 30 seconds, and then a 22 mL aliquot will be removed and transferred to a glass bottle containing 198 mL of sterile distilled water. This dilution (1:100) will be vortexed for 30 seconds, after which a 20 mL aliquot will be removed and added to 180 mL of sterile distilled water to generate 1:1000 dilution samples. Each 200-mL graywater sample (included an undiluted sample) will be vortexed for 30 seconds and immediately divided into 2 100-mL samples, contained in 100 mL sterile glass bottles. The contents of one Colilert pack will be added to one bottle, and the contents of one Enterolert pack will be added to the second bottle of each sample. Bottles will be capped and shaken until contents are dissolved. Each sample/reagent mixture will be poured into a Quanti-Tray®/2000 and sealed by the IDEXX Quanti-Tray® Sealer. Sealed trays will be placed in a 35°C ± 0.5°C incubator for 24 hours. Results will be read according to the Result Interpretation table provided by IDEXX. The number of positive wells will be counted and the MPN of total coliforms, *E. coli*, and enterococci will be calculated based on the provided MPN table.

Table 1. Methods for Analysis of Standard Wastewater Parameter and Metals

Parameter	Method	Description
BOD	SM 5210 B	5 day BOD test
COD	SM 5220 D	Closed Reflux, Colorimetric Method
TOC	SM 5310 B	High Temperature Combustion
TSS	SM 2540 D	TSS Dried at 103-105C
TDS	SM 2540 C	TDS Dried at 180C

pH	SM 4500 H	Electometric Method
ORP	SM 2580	ORP Electrode
conductivity	SM 2510	Conductivity Meter
TN	SM 4500 N	In-Line UV/Persulfate Digestion and Oxidation with Flow Injection Analysis
NH_4-N	SM 4500 NH3 D	Ammonia-Selective Electrode Method
NO_3-N	SM 4110 B	Ion Chromatography
NO_2-N	SM 4110 B	Ion Chromatography
TP	SM 4500-P C	Vanadomolybdophoshoric Acid Colorimetric Method
PO_4	SM 4110 B	Ion Chromatography
hardness	SM 2340 C	EDTA Titrimetric Method
metals	SM 3120	Metal by Plasma Emission Spectroscopy

Soil Samples

Soil samples for surfactant and antimicrobial analyses: An extraction step will be required for analysis of surfactants and antimicrobials in all 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%. Subsequent analysis will be conducted by LC-MS. This method may be optimized for analysis of surfactants in soil samples. Prior to our first sample collection, the research team will run experiments in the lab with soils having similar characteristics to that expected to be collected at study sites. Known concentrations of surfactants will be injected into these soil samples and the research team will determine the recovery rate of these surfactants based on the described extraction method. If needed, the method will be modified to improve the recovery rate. 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.

Soil samples for indicator organism analyses: Fresh soil samples will be immediately analyzed in Dr. Stromberger's laboratory 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. Each soil depth increment (0-15, 15-30, and 30-100 cm depths) will be analyzed to assess the potential for downward movement of pathogen indicators, and thus potential for groundwater contamination. Soil samples (22.2 g) will be diluted ten-fold in 199.8 mL of sterile physiological saline (0.85% NaCl). The slurry will be sonicated for 2 minutes, then vortexed at maximum speed for 2 minutes to disperse soil particles and dislodge cells from soil particles. After a 5 minute settling period, 22 mL of this 1:10 dilution will be transferred to a sterile glass bottle containing 198 mL sterile PBS (1:100 dilution). The 1:100 soil suspension will be vortexed for 30 seconds, and after a 30-second period, 20 mL will be transferred to a third glass bottle containing 180 mL sterile PBS (1:1000 dilution). Each 200-mL soil suspension will be vortexed for 30 seconds and immediately divided into 2 100-mL samples, contained in 100 mL sterile glass bottles. The contents of one Colilert pack will be added to one bottle, and the contents of one Enterolert pack will be added to the second bottle of each sample. Bottles will be capped and shaken until contents are dissolved. Each sample/reagent mixture will be poured into a Quanti-Tray®/2000 and sealed by the IDEXX Quanti-Tray® Sealer. Sealed trays will be placed in a 35°C ± 0.5°C incubator for 24 hours. Results will be read according to the Result Interpretation table provided by IDEXX. The number of positive wells will be counted and the MPN of total coliforms, *E. coli*, and enterococci will be calculated based on the provided MPN table.

Soil subsamples shipped to EMLab Pand K Laboratories in San Bruno, CA will be immediately analyzed upon arrival for *Clostridium perfringens* according to the plate count method specified in the Bacteriological Analytical Manual (FDA BAM), using a clostridial agar medium called tryptose-sulfite-cycloserine (TSC) agar (egg yolk free). With aseptic techniques, 25.0 g of each soil sample will be added to a sterile blender jar, along with 200 mL peptone dilution fluid (1:10 dilution). The slurry will be homogenized 1-2 min at low speed to obtain a uniform homogenate with as little aeration as possible. Using the 1:10 dilution prepared above, serial dilutions from 10^{-1} to 10^{-6} will be made by transferring 10 mL of appropriate suspension to 90 mL peptone diluent blanks. Each dilution will be mixed thoroughly by gently shaking before each transfer. To prepare agar plates, 6-7 mL of TSC agar without egg yolk will be poured into each of ten 100 x 15 mm petri dishes per soil dilution sample and spread evenly on bottom by rapidly rotating dish. When the agar has solidified, plates will be labeled with soil sample identification and dilution series. Then, 1 mL of each dilution will be added to the center of duplicate agar plates. An additional 15 mL TSC agar without egg yolk will be poured into each dish and mixed with inoculum by gently rotating dish. When agar has solidified, plates will be placed in upright

positions in an anaerobic jar. Anaerobic conditions will be established, and the jar(s) will be placed in a 35°C incubator for 20-24 h. After incubation, plates will be removed from the anaerobic jar, and plates containing 20-200 black colonies will be selected for counting.

Soil samples for physical and chemical analyses: Soil texture (particle size) will be determined on each sample using the hydrometer method described by Gee and Bauder (1986). Soil bulk density will be calculated based on the volume of the removable soil core liner (based on sampling depth), the mass of the field-moist core, and the gravimetric moisture content of the soil core, determined on a subsample. Air-dried soil samples (~500 g) will by analyzed by CSU's Soil, Water and Plant Testing Laboratory for multiple chemical properties. Soil samples from the surface (0-20 cm depth, where the research team expect graywater to have the greatest impacts) will be analyzed for pH, electrical conductivity (EC), organic matter, total C, total N, extractable NH_4-N, NO_3-N, P, B, Ca/Mg/K for effective cation exchange capacity (CEC_e), and Na for sodium adsorption ratio (SAR; calculated by the ratio of Na to Ca+Mg). Soil pH will be determined by the saturated paste method (Method 21a of USDA Handbook No. 60, 1954) and soil EC will be determined by the saturated paste method of Rhoades (1996). Percent organic matter will be determined by the modified Walkley-Black method described by Nelson and Sommers (1996). Total C and N will be determined using a LECO CHN-1000 automated analyzer (LECO, St. Joseph, MI) according to the protocols of Nelson and Sommers (1996). Exchangeable soil NH_4-N and NO_3-N were extracted in 2 M KCl according to Mulvaney (1996) and analyzed on a Perstorp Enviroflow flow injector (Perstorp Analytical, Inc., Silver Spring, MD). The method of Kuo (1996) will be used for colorimetric determination of Mehlich III extractable P, K, Zn, Mn, Fe, and Cu. Concentrations of Ca, Mg, Na, and K will be 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 and CEC_e determination. Boron will be measured in soil samples with the hot water extraction method according to Gupa (1967). Air-dried soil samples from the deeper depths (20-60 cm and 60-100 cm depths) will be analyzed for EC, NO_3-N, B, and SAR according to the methods listed above to determine salt and N leaching potential and therefore potential groundwater impacts. Analytical methods for soil chemical and physical analyses will be done according to procedures found in the Quality Assurance Plan for the CSU Soil, Water, and Plant Testing Laboratory (see appendix). The appendix provides the Quality Assurance Plan for Soil Testing@. Balances used for weighing biomass will be calibrated on a daily basis adhering to existing lab QC procedures. Check weights are routinely used to test balance performance. Calibration procedures will follow the protocols found in the Quality Assurance Plan for the CSU Soil, Water, and Plant Testing Laboratory (see appendix).

Plant Samples

Prior to analyses, leaves will be separated based on age and be subjected to tissue analysis. The research team will oven dry plant biomass samples at 70EC to constant mass, and then the research team will store them at a designated storage site at CSU. After the biomass determinations, dried samples will be ground in a Wiley mill. Approximately 1 g of screened and

dried sample will be used for ion analysis (Na, Ca, Mg, K, B, and other metal ions) by inductively-coupled plasma atomic emission
spectrophotometry (ICP-AES). Chloride content will be analyzed by a Cl^- selective electrode. Analytical methods for plant-tissue analyses will be done according to procedures found in the Quality Assurance Plan for the CSU Soil, Water, and Plant Testing Laboratory (see appendix). The appendix provides the AQuality Assurance Plan for Soil Testing@. Balances used for weighing biomass will be calibrated on a daily basis adhering to existing lab QC procedures. Check weights are routinely used to test balance performance. Calibration procedures will follow the protocols found in the Quality Assurance Plan for the CSU Soil, Water, and Plant Testing Laboratory (see appendix).

Greenhouse Plant, Leachate and Soil Samples

Plant visual health and growth will be measured monthly based on density, dieback from tip, foliage color (chlorosis), and presence of insects or disease. To determine growth (vigor), plant size (height and diameter) will be measured. Bloom will be measured by counting the number of blooms on representative plants and estimating overall percent bloom. The percent foliar burn (leaf scorch and necrosis) will be estimated visually. Average leaf size will be determined by measuring with LI-COR 3100 leaf area meter. Tissue analysis will include quantification of chloride, boron, sodium, potassium, magnesium, nitrogen, phosphorus at the termination of the experiment, using methods described above for household plant samples.

The greenhouse experiments will be conducted over a period of 1 year, and leachate will be collected in three of the four replicates for each plant and analyzed four times during this duration (every 3 months) for analysis of TOC, TP, TN, NH_4^+, NO_x, hardness, conductivity, linear alkyl benzene sulfonate, alcohol ethoxylate, and alkyl ethoxy sulfate using methods described above for graywater samples. At the time the last set of leachate samples are collected, soil will be collected from the plant pots to measure accumulation of surfactants, nutrients, and boron, using methods described above for soil sample analysis. However, TP in soil samples will be extracted by the AB-DTPA method developed by Soltanpour and Schwab (1977).

B5: Quality Control Requirements

Graywater and Greenhouse Leachate Sample Blanks

Applications: Analysis of COD, TOC, TN, NH_4-N, NO_3-N, NO_2-N, TP, PO_4, metals, surfactants, and antimicrobials
Procedure: Blanks samples consisting of DI water will be analyzed by each method. When instruments such as IC, TOC/TN, ICP, or LC-MS are utilized, blank samples will be tested after analysis of every 10 samples.
Acceptance Criteria: The analyte of interest should be below the lower limit of detection for the method applied in blank samples.

Corrective Action: When blank samples are below the lower limit of detection for the applied method, the concentration of analyte present in samples will be calculated by subtraction of the concentration measured in the blank from the concentration measured in the sample. When blank samples are above the lower limit of detection for the applied method, sample analysis will be terminated and the instrument will be cleaned. Columns will be replaced as needed. Samples will not be analyzed until the concentration of analyte in blanks is lower than the low detection limit.

Graywater and Greenhouse Leachate Sample Replicates

Applications: Analysis of COD, TOC, TSS, TDS, pH, ORP, conductivity, TN, NH_4-N, NO_3-N, NO_2-N, TP, PO_4, metals, surfactants, hardness, and antimicrobials
Procedure: Duplicate or triplicate samples of a given analyte will be analyzed each time that a batch of samples is analyzed. A minimum of 5 % of the samples analyzed will be duplicates or triplicates for each sample run.
Acceptance Criteria: Measurement of replicate samples should not vary by more than 10%.
Corrective Action: If measurement of replicate samples varies by more than 10%, samples are initially reanalyzed to determine whether instrument failure was the cause. If replicate samples still vary by more than 10%, samples are completely reanalyzed beginning with either an extraction or digest.

Graywater and Greenhouse Leachate Sample Checks

Applications: Analysis of COD, TOC, TN, NH_4-N, NO_3-N, NO_2-N, TP, PO_4, metals, surfactants, and antimicrobials
Procedure: Samples of known concentration will periodically be tested each time that a batch of samples is analyzed. A minimum of 5 % of the samples analyzed will be sample checks for each run.
Acceptance Criteria: Measurement of known concentration samples should be outside of the known value by more than 10%.
Corrective Action: If check samples are outside of the known value by more than 10%, samples are initially reanalyzed to determine whether instrument failure was the cause. If check samples are still outside of the known value by more than 10%, samples are completely reanalyzed beginning with either an extraction or digest.

Graywater and Greenhouse Leachate Sample Matrix Spikes

Applications: Analysis of TN, , NO_3-N, NO_2-N, PO_4, surfactants, and antimicrobials (chromatography methods)
Procedure: Tested samples are spiked with a known concentration of analyte. This will be conducted once at least every 3 hours of instrument operation.
Acceptance Criteria: The concentration measured in the sample should be the same as a nonspiked sample plus the amount spiked within 10%.
Corrective Action: In the case that the concentration measured in the spiked sample should be the same as a nonspiked sample plus the amount spiked within 10%, the most likely cause is that the sample peak was incorrectly identified. In this case, a complete set of standards will be run

again to evaluate the time of elution for various analytes. Any samples that may have been misidentified will be analyzed again.

Graywater and Greenhouse Leachate Sample Internal Standards

Applications: Analysis of surfactants and antimicrobials (LC-MS methods)
Procedure: Known values of specified internal standards are added to standards and samples.
Acceptance Criteria: The recovery rate of the analyte of interest is calculated and should be more than 60%.
Corrective Action: When the recovery rate of the analyte is more than 60%, measured values of analyte are corrected to account for the recovery rate. When the recovery rate is less than 60%, sample extraction and concentration procedures are repeated until an acceptable recovery rate is achieved.

Indicator Organism Quality Control (Graywater and Soil Samples)

The following quality controls will be included for each batch of Colilert tests for E. coli and total coliforms: 1) one Quanti-Cult™*** *E. coli* (positive control), 2) one Quanti-Cult *Klebsiella pneumoniae* (total coliform positive control), and 3) one Quanti-Cult *Pseudomonas aeruginosa* (noncoliform negative control).

Soil and Plant Samples

Analysis of surfactants and antimicrobials in soil extractions will follow the same QA/QC procedures as listed above for graywater and greenhouse leachate analysis. For soil microbial analyses other than indicator analyses, no reference standard is required because all samples will be processed for each individual analysis at the same time. See the Appendix for quantitative criteria for QA objectives, assessment procedures, and QC checks and frequencies. Plant tissue analysis will be conducted in the Soil, Water, and Plant testing Lab at Colorado State University. This lab is currently accredited for soil and plant analysis by the North American Proficiency Testing Program. The QC/QA for soil samples and plant tissue analysis will include regular instrument maintenance, periodic calibration, and running duplicate samples and comparing results. By periodically analyzing standards (samples with known values) during sample runs, the research team can determine if the results are reproducible and accurate. All of the procedures are documented and maintained by our standard operating procedures. A minimum of 12% of the samples analyzed will be quality control samples comprised of blanks, references and duplicates.

B6: Instrument/Equipment Testing, Inspection, and Maintenance Requirement

Field equipment will consist of scissors or knives to harvest plant biomass, and augers to take soil samples. There are no testing, inspection, or maintenance requirements for these items.

Graywater and Greenhouse Leachate Samples

TOC/TN Analyzer: All consumables are replaced as recommended by the instrument manufacturer. A maintenance calendar is placed near the TOC/TN analyzer listing required maintenance activities, the last date performed and the subsequent date that the activity is to be performed. This maintenance calendar is checked by the lab manager and lab PI to ensure that activities have been performed as scheduled.

IC: Daily maintenance involves replenishing eluent and regenerate, checking tubing for clogs loose connections and bubbles, and checking pump pressure. The columns are checked for contamination or clogging.

ICP: ICP maintenance is performed daily, where the torch assembly and nebulizer are cleaned with acids or distilled water. Pump tubing is replaced once per week. The entire machine is vacuumed and wiped down once per week. Drain tubing is replaced once per month.

LC-MS: Prior to and directly following each use of the LC-MS, the instrument tubing and column are cleaned with DDI water and organic solvents. The MS portion of the instrument is taken apart and cleaned with DDI water and organic solvents before each use of the instrument. The system pressure is checked to ensure that clogs are not present. The instrument is tuned per manufacturer guidelines once every two weeks.

Soil and Plant Samples

See the Appendix for instrument/equipment testing, inspection, and maintenance requirement criteria.

B7: Instrument/Equipment Calibration and Frequency

None of the field equipment requires calibration

Graywater and Greenhouse Leachate Samples

TOC/TN Analyzer: The TOC/TN analyzer is calibrated at the beginning of each run with known concentrations of organic carbon and nitrogen. Five calibration levels are used for each instrument run. Anhydrous primary-standard -grade potassium biphthalate is used for calibration of organic carbon, anhydrous sodium carbonate is used for calibration of inorganic carbon, and sodium nitrate or potassium nitrite are used for calibration of total nitrogen.

IC: A calibration curve is run at the beginning and end of each IC run. Five calibration levels are used for each instrument run. Anions used for calibration include sodium nitrate, potassium nitrite, and trisodium phosphate.

ICP: Calibration is run according to the Soil, Water, and Plant Testing Laboratory (see Appendix)

LC-MS: A calibration curve is generated at the beginning, end, and every 3 hours during operation of the LC-MS. Five calibration levels are used at each time of calibration. High purity surfactants will be obtained from Procter and Gamble for generation of calibration curves. High purity antimicrobials are available from Sigma-Aldrich.

Meters: Meters for analysis of pH, ORP, conductivity, and NH_4-N analysis will be calibrated each time samples are analyzed.

Soil and Plant Samples

See the Appendix for instrument/equipment calibration and frequency criteria.

B8: Inspection/Acceptance Requirements for Supplies and Consumables

All sample containers will be inspected for closure and holes prior to transportation to CSU in order to avoid contamination. Throughout laboratory analyses procedures, containers will be also inspected to ensure complete closure and lack of holes, and if necessary containers will be replaced.

B9: Data Acquisition Requirements (Non-Direct Measurements)

The research team will not be collecting data from existing data sources.

B10: Data Management

Field data will be kept in field notebooks and manually transferred to an MS Excel spreadsheet. The data will be reviewed by the principal investigator prior to statistical analyses.

All raw data generated during laboratory analyses will be recorded directly into laboratory notebooks. The raw data will then be recorded into Microsoft Excel spreadsheet files to be stored electronically.

All univariate data will be analyzed using SAS (SAS Institute, v.9.1, Cary, NC), with an α value of 0.05 for significance determination. For the existing household study, a paired t-test will be performed on each data set to compare the effects of graywater versus potable water on plants and soils (with the treatments paired at each location). The paired design will account for location-specific factors that might influence the outcome of the experiment that are not part of the treatment (e.g., soil texture, soil organic matter content or plant type) thus providing greater sensitivity for detecting graywater effects. The new household study will be analyzed as a repeated measures paired t-test since locations will be repeatedly sampled over time; this design will examine the temporal response of plants and soils to irrigation with graywater versus potable water. For data collected at various soil depths, a split-plot statistical design will be employed to test for significant changes in irrigation water-borne constituents with soil depth, using irrigation

water type (gray- versus potable water) as the main effect and soil depth increment as the split effect. When significant, depth increment means will be separated by the least significance difference (LSD) method.

Microbial community EL-FAME data will be analyzed with the PC-ORD software to test for differences in microbial community composition between graywater and potable water-treated soils. EL-FAMEs will be checked for normality and arcsine-square root transformed if nonnormal. All EL-FAMEs will be expressed on a relative mol percent basis prior to multivariate
analysis by principal components analysis (PCA) or non-metric multidimensional scaling, whichever is appropriate based on EL-FAME normality distribution. By expressing EL-FAME data as relative percents, rather than concentrations, the analysis of community composition will not be affected by differences in biomass size between the treatments or among the locations.

All data Excel spreadsheets and SAS and PC-ORD files will be downloaded onto two CDs. Laboratory notebooks are kept indefinitely, and all downloaded electronic files will be kept at least 5 years after the data has been published. Hard (paper) copies of all electronic files will also be kept in a file for at least 5 years following publication of data. Each Project Investigator will review the raw data in the laboratory notebook to ensure that all values were correctly recorded by hourly students into the electronic spreadsheets. Any data transformations conducted by the Excel software will be double checked for accuracy by hand calculating the transformations of several samples. All software programs are routinely upgraded when software changes occur.

Group C: Assessment and Oversight

C1: Assessments and Response Actions

See the appendix for procedures for corrective actions and parties responsible for implementing corrective actions. Individuals involved in sampling will meet prior to sampling to discuss procedures to ensure consistency.

C2: Reports to Management

The research team will provide WERF with reports and updates updates according to the schedule below. Reports will document graywater quality and effects of graywater irrigation on soils and plants relative to irrigation with potable water. In addition, annual reports will present the status of each project goal and milestone progress, including adjustments to completion dates if necessary. Results and conclusions will be summarized in a final report for WERF by February 14, 2011.

Year One (2/15/2008-2/14/2009)	Date Due:
Site Selection and Sampling Plan Report	6/6/2008
Revised Site Selection and Sampling Plan Report	7/7/2008
Progress Report	10/15/2008
Annual Report	2/1/2009

Year Two (2/15/2009-2/14/2010)	Date Due:
Letter of Progress	6/15/2009
Progress Report	10/15/2009
Annual Report (Greenhouse Studies)	2/1/2010

Year Three (2/15/2010-2/14/2011)	Date Due:
Progress Report	6/15/2010
Letter of Progress	10/15/2010
Draft Final Report	12/15/2010
Final Report	2/14/2011

Group D: Data Validation

D1: Data Review, Validation, and Verification Requirement

If outliers or analytical values which exceed 10% of the duplicate value or reference sample occur, then the samples will be analyzed again. Operator technique will be reviewed, the instrument will be examined for malfunctions, and glassware will be checked for cleanliness. Data will be examined by Project Investigators to ensure that all values were transcribed properly by hourly students into electronic files. A minimum of 10% of the transcribed data points will be checked to ensure accurate data transfer.

D2: Validation and Verification Methods

Field and laboratory data will be recorded in MS Excel computer spreadsheets. The research team will print all data as a backup and will verify all data prior to statistical analyses. Verified data will be imported directly into SAS Version 9.1 (or later version) statistical software to complete the statistical analyses. The research team will also transfer all data to a CD-RW as an additional backup.

D3: Reconciliation with User Requirements

Financial support from WERF will directly result in the collection and public dissemination of information regarding short- and long-term effects of graywater irrigation on landscape plants and soils. Specifically, WERF funds for this research will produce quarterly reports, two annual progress report, one final report, a graduate student thesis, and a several presentations at national professional society meetings and refereed journal publications.

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APPENDIX B

PLANT ANALYSIS

Table B-1. Shoot Mineral Content of Different Landscape Plants Grown on Sites under Long-Term (more than 5 years) Graywater (GW) Irrigation vs. Freshwater (FW) Irrigation.

State	Plant	Treatment	Shoot Mineral Content (mg/kg)											
			Cl	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	Si	K/Na
AZ	Saltbush	GW	N/A	55	11611	10	80	32805	4843	81	86	2413	252	383
AZ	Saltbush	FW	19295	60	14301	11	109	39040	4908	95	73	1792	348	533
AZ	Mallow	GW	13674	61	9231	12	190	16875	1860	56	144	1713	645	117
AZ	Mallow	FW	6908	124	36906	12	265	9835	2147	94	70	2387	936	140
AZ	Honey mesquite	FW	23144	19	13441	12	45	8235	1882	51	66	1532	92	125
AZ	Honey mesquite	GW	13095	22	17326	6	81	7200	2485	52	94	1628	186	77
AZ	Tobacco tree	GW	30471	68	45521	12	53	6535	3854	83	206	2226	298	32
AZ	Tobacco tree	FW	44842	83	50746	21	58	14365	2342	171	110	4813	305	130
AZ	Desert daisy	GW	15564	57	16451	11	317	28045	4097	85	68	2209	1179	410
AZ	Desert daisy	FW	9914	32	19846	12	139	27270	4014	55	48	2082	445	570
AZ	Hackberry	GW	2311	93	33211	5	54	6560	3896	124	52	1117	2250	126
AZ	Hackberry	FW	872	29	26191	8	82	11160	3104	89	80	1549	1184	139
CO	Scotch pine	GW	431	27	5756	2.6	70.4	4595	13570	17.5	27	1016	312	174
CO	Scotch pine	FW	324	100	8163	14.0	65.0	3274	1836	43.8	114	842	478	29

State	Plant	Treatment	Shoot Mineral Content (mg/kg)											
			Cl	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	Si	K/Na
CO	Juniper	GW	233	42	10595	8.3	60.7	6105	2875	18.0	31	1845	737	196
CO	Juniper	FW	169	36	10272	6.1	109.4	5031	2732	27.7	31	1846	551	163
CO	Mugho pine	GW	399	74	4387	2.9	46.6	3692	1337	13.9	37	1049	340	100
CO	Mugho pine	FW	471	55	6058	3.5	80.8	4151	1171	47.0	29	1071	290	145
CO	Euonymus	GW	2247	66	29590	7.0	78.1	11650	4761	23.8	220	3189	3080	53
CO	Euonymus	FW	1522	48	31560	10.0	104.0	9776	5116	32.8	78	2537	1502	126
CO	Rose of Sharon	GW	2731	92	35130	17.1	92.0	12500	9659	27.7	86	2289	783	145
CO	Rose of Sharon	FW	2447	97	34250	12.8	74.4	10910	10100	46.6	77	2487	738	142
CO	Mum (purple)	GW	19979	54	15830	20.4	80.0	34160	4513	46.8	142	4311	2970	240
CO	Mum (white)	FW	15482	53	12940	13.9	105	28920	5167	28.1	1027	3772	3876	28
CO	‘Himalayan Border Jewel’	GW	3687	66	29660	12.3	76.8	11290	4690	23.0	203	3160	3049	56
CO	‘Himalayan Border Jewel’	FW	6358	47	9929	4.3	77.0	17260	5143	30.8	107	1859	966	162
TX	Velvet Ash	GW	375	21	22280	5.2	53.0	7056	2233	52.8	73	1369	1286	96
TX	Velvet Ash	FW	657	25	17920	7.5	68.6	8917	2443	48.9	150	1284	1295	60
TX	Velvet Ash	FW	472	16	14200	3.4	65.7	8525	2352	36.4	50	1574	870	171
TX	Velvet Ash	GW	798	42	20570	8.4	61.6	8950	2250	49.6	96	1385	1068	94
TX	Bearded Iris	GW	5527	30	24720	3.3	67.8	30320	1486	21.2	243	1965	1076	125

State	Plant	Treatment	Shoot Mineral Content (mg/kg)											
			Cl	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	Si	K/Na
TX	Bearded Iris	FW	4436	27	26730	1.6	41.8	26360	1130	10.8	101	1159	924.9	260
TX	St. Augustine	FW	15974	7.7	8016	8.5	119.9	21600	2027	52.9	9403	2267	2330	2.3
TX	St. Augustine	FW	23359	6.4	7477	7.5	97.2	20290	2007	32.0	11440	2314	2764	1.8
TX	St. Augustinegrass	GW	25805	7.4	6862	9.1	117.6	17375	1872	51.2	12845	3038	2670	1.4
TX	St. Augustinegrass	GW	19389	6.5	6482	7.6	97.3	15420	1797	34.1	10770	4339	2972	1.4
TX	Privet	GW	4019	33	19450	8.6	71.6	19910	3217	121	17	1923	1776	1176
TX	Privet	FW	3895	25	26060	6.0	43.6	5353	2148	180	232	861	1512	23
CA	Hass Avocado	GW	5840	10	10810	5.1	99.8	3930	5542	114	79	1232	1657	50
CA	Hass Avocado	FW	14220	41	11790	10.4	95.5	15640	6133	146	89	1461	3359	176
CA	Wintercreeper Euonymous	GW	3570	77	26570	8.2	96.2	17935	3374	34.5	961	4345	2304	19
CA	Wintercreeper Euonymous	FW	1868	72	20550	11.7	108.7	21540	2831	22.4	450	4324	2268	48
CA	Yellow Bush Daisy	GW	51987	120	13800	7.0	149.0	16790	4245	112	22430	2195	2853	0.75
CA	Yellow Bush Daisy	FW	27652	62	21140	2.8	231	15750	4730	107	7335	1345	2277	2.1
CA	Yellow Bush Daisy	FW	11527	64	15370	4.1	378	14510	4000	81	6386	2249	4068	2.3

State	Plant	Treatment	Shoot Mineral Content (mg/kg)											
			Cl	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	Si	K/Na
CA	California Valeriana	GW	23359	74	15700	12.7	64.4	26790	6532	52.6	3890	2034	4804	6.9
CA	California Valeriana	FW	11869	45	14578	8.4	86.9	15360	4914	27.0	3751	3195	3281	4.1
CA	Lemon	GW	8246	121	27180	3.6	111.5	8903	4257	14.9	102	1467	2758	88
CA	Plum	GW	258	27	20950	7.3	78.4	11510	3838	43.4	77	2260	496	150
	For samples collected in September 2010													
CO	Scotch pine	GW	661	72	6275	31	53	5600	3530	15.5	34	1545	6	211.5
CO	Scotch pine	FW	863	74	9425	3	70	4355	3830	46.5	239	2035	4.5	19
CO	Juniper	GW	458	55	10925	24	101	7650	5225	29	31	3045	6.5	274
CO	Juniper	FW	433	54	11400	8	56	7025	5280	22	28	2845	8	273
CO	Mugho pine	GW	608	58	3675	6	63	3805	2800	7.5	23	1125	8.5	167
CO	Mugho pine	FW	366	41	4515	3	70	3410	3115	32.5	25	1205	11	143
CO	Euonymus	GW	1754	57	37000	19	86	12600	6450	21.5	197	4595	21	64
CO	Euonymus	FW	1214	34	37062	5	91	10553	6520	22	52.5	3158	20.5	199
CO	Rose of Sharon	GW	3338	102	45250	18	90	17200	10500	23.5	54.5	3035	13	316
CO	Rose of Sharon	FW	1118	118	55300	8	77	8800	11750	45.5	44.5	3170	14	198
CO	Mum (purple)	GW	7339	41.5	19200	28	98	31150	7300	50.5	279	8650	33	111
CO	Mum (white)	FW	27077	71	19850	136	87	35900	7800	141.5	297	5850	38.5	121
CO	Himalayan Border Jewel	GW	3895	52	8700	10	64	11650	5900	40.5	216	1755	16.5	54

State	Plant	Treatment	Shoot Mineral Content (mg/kg)											
			Cl	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	Si	K/Na
CO	Himalayan Border Jewel	FW	2830	36	10450	3	48	13950	6100	20.5	147	1880	9	95

Table B-2. Comparison of Mineral Content (mg/kg) of Landscape Plants Sampled at the AZ New Household. Samples Were Collected for graywater (GW) and Freshwater (FW) Irrigated Plants in 2008, 2008, and 2010.

State	Plant	Water	Cl	B	Ca	Cu	Fe	K	Mg	Mn	Na	P
							mg kg^{-1}					
						2008-Baseline						
AZ	Bermudagrass		5534	10	7516	7.1	188	22060	2728	77	845	4027
AZ	Lemon grass		6398	39	6972	3.1	44	19860	2490	16	251	1462
AZ	Orange		115	129	27400	6.4	45	14790	2607	18	86	1577
AZ	Lemon		342	244	34570	6.2	76	14290	2430	30	36	984
						2009- June						
AZ	Bermudagrass	FW	5328	6	5286	5.8	55	15530	1870	58	488	5466
AZ	Bermudagrass (mowed)	FW	4381	8	5416	4.5	102	13970	1808	66	607	4640
AZ	Bermudagrass	GW	14716	9	8271	6.5	67	17550	1821	58	1418	3122
AZ	Lemon grass	FW	13440	174	35436	8.6	37	20290	2055	15	125	1501
AZ	Lemon grass	GW	13324	28	4761	7.8	26	20395	1921	25	751	2178
AZ	Lemon	FW	1019	65	8076	3.0	23	18985	2640	78	598	2235
AZ	Lemon	GW	460	85	36531	5.0	47	13815	2254	11	70	1450
AZ	Peach	FW	2146	56	14496	6.6	62	17525	3644	22	155	2455
AZ	Peach	GW	1892	56	22311	8.4	63	22065	3536	31	71	2401
AZ	Black eyed susan	FW	41517	146	53746	13.9	49	22190	4408	112	619	2391
AZ	Black eyed susan	GW	36581	288	63596	9.6	56	16895	4952	220	173	2395
						2010-January						
AZ	Bermudagrass	GW	16737	9	4578	3.2	75	5878	1913	89	2171	1462
AZ	Bermudagrass (mowed)	FW	5685	6	2717	11.1	183	3415	1560	43	590	3683
AZ	Lemon grass	GW	20417	12	4550	4.8	43	9875	5027	65	328	1052
AZ	Lemon grass	FW	18575	10	3097	3.3	36	9634	2580	77	447	1461
AZ	Lemon	GW	5030	121	16400	10.3	63	8558	2906	12	124	1637
AZ	Lemon	FW	1593	112	13350	9.9	42	7201	1739	16	59	1217

State	Plant	Water	Cl	B	Ca	Cu	Fe	K	Mg	Mn	Na	P
							mg kg^{-1}					
AZ	Peach	GW	8025	21	10700	11.7	87.8	9251	5494	46	54	2729
AZ	Peach	FW	4961	20	8545	6.3	93.5	10340	4552	37	43	2345
AZ	Canna	GW	5013	54	2972	1.4	53.3	15480	4448	216	2028	1500
AZ	Canna	FW	3209	49	2635	11.7	52.0	18040	4307	51	5678	1241
						2010-June						
AZ	Bermudagrass	GW	18879	6	3177	5.1	65.0	13975	2020	62	744	463
AZ	Bermudagrass (mowed)	FW	11405	7	3124	5.2	64.2	8565	2048	58	758	3252
AZ	Lemon grass	GW	1350	15	1973	10.2	29.2	12240	1854	44	154	2835
AZ	Lemon grass	FW	15512	12	2149	5.4	28.1	11945	1492	55	629	1634
AZ	Lemon	GW	941	73	16060	4.7	35.9	7210	2813	11	56	1366
AZ	Lemon	FW	1289	125	18110	5.5	54.8	7528	2680	20	60	1159
AZ	Peach	GW	8372	32	8642	10.3	75.0	10420	5403	16	67	1576
AZ	Peach	FW	3912	43	6066	6.3	68.9	12885	4558	26	75	2128
AZ	Canna	GW	20645	59	3071	6.3	51.1	22365	4026	317	1764	2206
AZ	Canna	FW	12288	55	2685	8.0	43.0	19060	3300	155	4334	1683
AZ	Black-eyed susan	GW	14325	220	25435	10.9	66.3	11264	7692	93	135	1836
AZ	Black-eyed susan	FW	12869	199	27870	9.4	48.6	10152	8487	140	177	2071
AZ	Rose	GW	6449	124	10615	7.1	43.1	6222	4416	68	71	2037
AZ	Rose	FW	6987	121	9889	4.8	50.4	7566	4509	102	373	1456

Table B-3. Comparison of Mineral Content (mg/kg) of Landscape Plants Sampled at the CA New Household. Samples Were Collected for graywater (GW) and Freshwater (FW) Irrigated Plants in 2008, 2010, and 2011.

State	Plant	Water	Cl	B	Ca	Cu	Fe	K	Mg	Mn	Na	P
							mg kg^{-1}					
					September 2008 (Baseline)							
CA	Pear		585	22	22390	3.7	78	11360	4246	91	109	1526
CA	Bermudagrass		3681	8	8348	2.2	66	8839	2396	87	214	2287
CA	Apple		6373	23	20800	3.3	80	4567	5751	43	96	1437
CA	Edible Fig		1954	169	33000	2.4	125	17890	9049	84	413	2148
					October 2010							
CA	Pear	GW	825	23	19280	5	77	14278	6958	70	76	2066
CA	Pear	FW	528	16	19530	4	56	6300	7220	33	103	1366
CA	Bermudagrass	GW	6769	3	4997	2	101	17640	3250	215	317	3425
CA	Bermudagrass	FW	3165	3	7850	2	73	10770	5830	133	221	2386
CA	Apple	GW	549	23	19398	4	70	8690	6620	46	98	3574
CA	Apple	FW	625	24	15580	4	130	11700	6765	45	101	3265
CA	Edible fig	GW	820	98	36340	2	116	12345	9300	74	389	1520
CA	Edible fig	FW	1788	130	37420	3	144	19730	10365	74	463	2187
CA	Mallow	GW	2856	99	29860	7	173	26430	8200	179	74	8445
CA	Mallow	FW	1822	71	35940	6	257	25270	9710	281	250	4157
					October 2011							
CA	Pear	GW	5812	7.7	6898	1.7	111	20655	2478	98	367	3227
CA	Pear	FW	511	25.9	22860	5.5	79	6508	5245	34	91	1842
CA	Bermudagrass	GW	2510	116	37830	1.5	104	13290	7570	93	493	1751
CA	Bermudagrass	FW	2451	124	35370	7.1	112	19740	9423	87	378	1602
CA	Apple	GW	839	25	22100	6	81	10159	4457	92	115	1921
CA	Apple	FW	450	16	23300	5	55	6255	4558	40	82	1443
CA	Edible fig	GW	389	29.4	17170	5.59	90.9	10290	4679	45	89	3152
CA	Edible fig	FW	637	19.9	13340	6.96	123	8045	4111	52	131	2593
CA	Mallow	GW	6747	62.6	27690	9.29	211	27220	6447	318	169	4584
CA	Mallow	FW	3883	75.8	31120	5.38	170	20780	6040	131	87	5002

Table B-4. Comparison of Mineral Content (mg/kg) of Landscape Plants Sampled at the CO Prototype Household. Samples Were Collected for graywater (GW) and Freshwater (FW) Irrigated Plants in 2010 and 2011.

State	Plant	Water	Cl	B	Ca	Cu	Fe	K	Mg	Mn	Na	P
						mg kg-1						
					September 2010							
CO	Blue mist spirea	GW	2010	44	25225	7	149	23665	4303	67	54	2804
CO	Blue mist spirea	FW	998	39	21725	22	99	18380	3740	20	45	9535
CO	Lavender	GW	2657	38	17660	19	118	17215	7110	29	47	2821
CO	Lavender	FW	3895	58	19325	9	159	16615	8035	17	58	2441
CO	Basket of gold	GW	234	71	40900	4	143	28175	5815	52	51	4549
CO	Basket of gold	FW	998	39	21725	22	99	18380	3740	20	45	9535
CO	Russian Sage	GW	3193	73	24560	15	93	29625	6320	52	77	3079
CO	Russian Sage	FW	1665	47	20565	11	140	32285	3942	25	65	2617
CO	Peony	FW	3459	81	28280	3	85	8955	4423	32	19	2056
CO	Iris	GW	4646	29	21373	3	65	29103	4715	16	62	2815
CO	Iris	FW	5515	40	23035	3	45	26450	3753	14	44	2255
						September 2011						
CO	Blue mist spirea	GW	1890	65	21830	6	100	30480	1723	54	60	3373
CO	Blue mist spirea	FW	1587	46	21230	18	93	21380	1621	21	48	7667
CO	Basket of gold	GW	324	88	54200	5	134	25640	3128	67	39	3837
CO	Basket of gold	FW	421	89	48210	2	183	33400	4072	52	77	5297
CO	Russian Sage	GW	4151	85	25430	8	82	33120	3020	53	68	2545
CO	Russian Sage	FW	2331	65	21910	8	119	34180	1893	37	53	2796
CO	Peony	FW	6448	72	26570	4	74	19710	3212	20	27	1335
CO	Peony	GW	5792	93	31230	4	66	8839	2609	22	22	1981

APPENDIX C

SOIL ANALYSIS

Table C-1 Soil Physical and Chemical Properties at the AZ Household with an Existing Graywater Irrigation System.

Date	Depth	Irrigation Type	Sand	Silt	Clay	Texture	CEC	Organic Matter	Total C	Total N	NH4-N	NO3-N	pH	EC
				(%)			meq 100 g^{-1}		(%)			mg kg^{-1}		$\mu S\ cm^{-1}$
Jun '09	0-15	Freshwater	78	12	10	Sandy loam	5.31	5.4	5.9	0.48	1.8	86.3	7.5	500
	0-15 recent		61	24	15	Sandy loam	4.18	2.4	4	0.17	0.6	23.4	7.5	500
	0-15 (0-2)		58	23	19	Sandy loam	3.03	1.5	2.6	0.08	0.6	34.9	7.6	1600
	0-15 (2-3)		42	28	30	Clay loam	2.98	1.6	3	0.09	0.9	10.5	7.9	500
	0-15 (3-4)	Graywater	61	19	20	Sandy clay loam	3.28	1.5	2.9	0.08	0.3	8	8	400

Date	Depth	Irrigation Type	SAR	B	P	K	Zn	Fe	Mn	Cu
						mg kg^{-1}				
Jun '09	0-15	Freshwater	1	0.29	1.4	471	61.4	83	126	3.1
	0-15 recent		1.9	0.31	2.7	507	17.8	72	236	3.9
	0-15 (0-2)		0	0.02	2.2	654	2	3.2	8	9.5
	0-15 (2-3)		0.2	0.01	1.3	371	2.1	3.6	5.4	8.5
	0-15 (3-4)	Graywater	0.3	0.01	2.2	360	1.4	3.3	3.3	9.7

Table C-2 Soil Physical and Chemical Properties at the CA Household with an Existing Graywater Irrigation System.

Date	Depth	Irrigation Type	Sand	Silt	Clay	Texture	CEC	Organic Matter	Total C	Total N	NH_4-N	NO_3-N	pH	EC
			(%)				meq 100 g^{-1}		(%)		mg kg-1			µS cm^{-1}
	0-15	Freshwater	49	30	21	Loam	24.7	4.6	2.16	0.19	3.2	8.9	7.2	2000
Oct '08	0-15	Graywater	60	22	18	Sandy loam	25.9	3.2	1.31	0.13	2.5	4.4	7.4	1600

Date	Depth	Irrigation Type	SAR	B	P	K	Zn	Fe	Mn	Cu
					mg kg-1					
	0-15	Freshwater	2.9	0.38	87.6	393	6.3	331	106	5.7
Oct '08	0-15	Graywater	3.3	0.38	61	323	6.1	347	81	6

Table C-3 Soil Physical and Chemical Properties at the CO Household with an Existing Graywater Irrigation System. (Nd: not determined)

Date	Depth	Irrigation Type	Sand	Silt	Clay	Texture	CEC	Organic Matter	Total C	Total N	NH4-N	NO3-N	pH	EC
				(%)			meq 100 g^{-1}		(%)		mg kg-1			$\mu S\ cm^{-1}$
		Freshwater	36	28	36	Clay loam	21.8	2.9	2.2	0.12	6.9	2.2	7.5	500
	0-15	Graywater	39	27	34	Clay loam	20.5	1.8	1.6	0.11	4.9	1.6	7.6	500
		Freshwater	24	24	52	Clay	Nd	Nd	Nd	Nd	Nd	2.1	Nd	500
	15-30	Graywater	18	26	56	Clay	Nd	Nd	Nd	Nd	Nd	2.5	Nd	1100
		Freshwater	16	28	56	Clay	Nd	Nd	Nd	Nd	Nd	1.5	Nd	1200
Oct '09	30-100	Graywater	20	26	54	Clay	Nd	Nd	Nd	Nd	Nd	2.4	Nd	1100
		Freshwater	40	28	32	Clay loam	20.6	5.2	3.4	0.19	4.7	4.9	7.3	500
	0-15	Graywater	32	29	39	Clay loam	20.7	1.5	1.6	0.1	1.7	3.2	7.7	300
		Freshwater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	3.3	7.9	400
	15-30	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	3.4	8	400
		Freshwater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	3.8	8	400
Sep '10	30-100	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	3.1	7.6	400
		Freshwater	22	26	52	Clay	23.1	2.3	2.073	0.11	1.7	0.67	7.2	576
	0-15	Graywater	16	28	56	Clay	23.1	1.7	1.521	0.1	0.88	1.5	7.8	432
		Freshwater	14	26	60	Clay	Nd	Nd	Nd	Nd	Nd	1.3	8	300
	15-30	Graywater	14	26	60	Clay	Nd	Nd	Nd	Nd	Nd	0.8	8	400
Sep '11	30-60	Freshwater	4	38	58	Clay	Nd	Nd	Nd	Nd	Nd	0.7	8.1	400

Date	Depth	Irrigation Type	Sand	Silt	Clay	Texture	CEC	Organic Matter	Total C	Total N	NH4-N	NO3-N	pH	EC
				(%)			meq 100 g^{-1}		(%)		mg kg-1			$\mu S\ cm^{-1}$
	30-60		8	34	58	Clay	Nd	Nd	Nd	Nd	Nd	1.1	7.9	300
	60-100	Graywater	8	28	64	Clay	Nd	Nd	Nd	Nd	Nd	0.8	8.1	800

Table C-3 Soil Physical and Chemical Properties at the CO Household with an Existing Graywater Irrigation System. (Nd: not determined; continued)

Date	Depth	Irrigation Type	SAR	B	P	K	Zn	Fe	Mn	Cu
							mg kg-1			
		Freshwater	0.7	2	44	289	1.9	30.7	7.1	2.8
	0-15	Graywater	0.6	2	12	325	0.9	15.7	5.4	2.4
		Freshwater	2.3	2.1	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Graywater	1.3	2.5	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	2.8	2.1	Nd	Nd	Nd	Nd	Nd	Nd
Oct '09	30-100	Graywater	3.3	2.3	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	0.6	6.2	144	299	4.2	98	64	6.2
	0-15	Graywater	0.8	4.7	81	355	1.4	57	55	4.7
		Freshwater	1.3	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Graywater	1.9	Nd	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	1.9	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Sep '10	30-100	Graywater	2.2	Nd	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	0.3	0.68	27	208	3	62.9	48.5	2.49
Sep '11	0-15	Graywater	0.6	0.7	41	303	2.7	54.9	46.7	4.17

Date	Depth	Irrigation Type	SAR	B	P	K	Zn	Fe	Mn	Cu
					mg kg-1					
		Freshwater	0.8	0.94	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Graywater	1	0.95	Nd	Nd	Nd	Nd	Nd	Nd
	30-60	Freshwater	1.6	0.86	Nd	Nd	Nd	Nd	Nd	Nd
	30-60		0.7	0.63	Nd	Nd	Nd	Nd	Nd	Nd
	60-100	Graywater	3.1	0.89	Nd	Nd	Nd	Nd	Nd	Nd

Table C-4 Soil Physical and Chemical Properties at the TX Household with an Existing Graywater Irrigation System. (Nd: not determined)

Date	Depth	Irrigation Type	Sand	Silt	Clay	Texture	CEC	Organic Matter	Total C	Total N	NH4-N	NO3-N	pH	EC
				(%)			meq 100 g^{-1}		(%)		mg kg-1			$\mu S\ cm^{-1}$
Sep '08		Freshwater	43	26	31	Clay loam	45.5	2.8	1.78	0.13	6.9	9.9	7.5	500
	0-15	Graywater	47	24	29	Sandy clay loam	47.7	7.3	6.85	0.3	7.2	8	7.5	500
		Freshwater	33	31	36	Clay loam	Nd	Nd	Nd	Nd	Nd	13.6	7.8	400
	15-30	Graywater	10	28	62	Clay	Nd	Nd	Nd	Nd	Nd	3.5	7.8	400
		Freshwater	21	37	42	Clay	Nd	Nd	Nd	Nd	Nd	5.3	8.2	400
	30-100	Graywater	28	24	48	Clay	Nd	Nd	Nd	Nd	Nd	2.5	8	300
Oct '09		Freshwater	50	32	18	Loam	34.8	2.5	10.3	0.65	5.8	21.3	7.4	700
	0-15	Graywater	47	27	26	Sandy clay loam	34.5	4.5	6.8	0.4	5.7	20.6	7.4	700
		Freshwater	10	24	66	Clay	Nd	Nd	Nd	Nd	Nd	2	Nd	300
	15-30	Graywater	12	28	60	Clay	Nd	Nd	Nd	Nd	Nd	4.8	Nd	400
	30-46	Freshwater	8	24	68	Clay	Nd	Nd	Nd	Nd	Nd	3.2	Nd	300

	Graywater	4	28	68	Clay	Nd	Nd	Nd	Nd	Nd	2.9	Nd	500
	Freshwater	4	26	70	Clay	Nd	Nd	Nd	Nd	Nd	2.1	Nd	400
46-61	Graywater	8	24	68	Clay	Nd	Nd	Nd	Nd	Nd	2.5	Nd	600
	Freshwater	8	24	68	Clay	Nd	Nd	Nd	Nd	Nd	2.3	Nd	300
61-76	Graywater	8	24	68	Clay	Nd	Nd	Nd	Nd	Nd	1.7	Nd	400
	Freshwater	4	26	70	Clay	Nd	Nd	Nd	Nd	Nd	2.3	Nd	400
76-91	Graywater	6	24	70	Clay	Nd	Nd	Nd	Nd	Nd	2.1	Nd	500

Table C-4 Soil Physical and Chemical Properties at the TX Household with an Existing Graywater Irrigation System. (Nd: not determined; continued)

Date	Depth	Irrigation Type	SAR	B	P	K	Zn	Fe	Mn	Cu
						mg kg-1				
Sep '08	0-15	Freshwater	1	0.29	1.4	471	61.4	82.6	126	3.1
		Graywater	1.9	0.31	2.7	507	17.8	71.5	236	3.9
	15-30	Freshwater	1.5	26.3	0.6	309	15.9	63.4	106	3.7
		Graywater	1.4	24.5	0.6	313	6.4	57.8	137	5.4
	30-100	Freshwater	3.3	24.6	0.1	219	8.4	38.7	68.7	3.4
		Graywater	1.8	25.1	0	183	2	29	48.7	2.6
Oct '09	0-15	Freshwater	<0.1	6.1	2.8	389	36.6	14.5	18.2	3.4
		Graywater	1.1	8.8	1.1	366	19.1	18.1	7.7	3.7
	15-30	Freshwater	0.4	2.6	Nd	Nd	Nd	Nd	Nd	Nd
		Graywater	2	10	Nd	Nd	Nd	Nd	Nd	Nd
	30-46	Freshwater	0.7	2.4	Nd	Nd	Nd	Nd	Nd	Nd
		Graywater	2.4	9.2	Nd	Nd	Nd	Nd	Nd	Nd

	Freshwater	1.9	1.6	Nd	Nd	Nd	Nd	Nd	Nd		
46-61	Graywater	1.7	5.2	Nd	Nd	Nd	Nd	Nd	Nd		
	Freshwater	1.7	1.2	Nd	Nd	Nd	Nd	Nd	Nd		
61-76	Graywater	2.1	3.6	Nd	Nd	Nd	Nd	Nd	Nd		
	Freshwater	2.7	1.1	Nd	Nd	Nd	Nd	Nd	Nd		
76-91	Graywater	2.5	3.3	Nd	Nd	Nd	Nd	Nd	Nd		

Table C-5. Soil Physical and Chemical Properties at the AZ Household with a New Graywater Irrigation System. (Nd: not determined)

Date	Depth	Irrigation Type	Sand	Silt	Clay	Texture	CEC	Organic Matter	Total C	Total N	NH4-N	NO3-N	pH	EC
				(%)			meq 100 g^{-1}		(%)		mg kg-1			$\mu S\ cm^{-1}$
		Freshwater	43	41	16	Loam	29	3.2	1.7	0.17	3	2.6	7.9	1800
Oct '08	0-15	Graywater	45	41	14	Loam	31.3	4.7	2.5	0.21	6.2	36.2	7.5	600
		Freshwater	Nd	Nd	Nd	Nd	7.66	2	1.9	0.16	6.1	7.2	7.6	800
	0-15	Graywater	59	Nd	16	Nd	5.4	4	2.2	0.14	6.5	11.3	7.4	1000
		Freshwater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	2.3	Nd	500
	15-30	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	5.4	Nd	600
		Freshwater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	1.4	Nd	600
Jun '09	30-100	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	3.4	Nd	700
		Freshwater	66	22	12	Sandy loam	17.5	2.4	1.6	0.19	0.6	10.8	7.6	600
	0-15	Graywater	66	21	13	Sandy loam	18	4	2.8	0.27	4.1	54.5	7.3	2000
Jan '10	15-30	Freshwater	36	36	28	Clay loam	Nd	Nd	Nd	Nd	Nd	1.5	Nd	500

Date	Depth	Irrigation Type	Sand	Silt	Clay	Texture	CEC	Organic Matter	Total C	Total N	NH4-N	NO3-N	pH	EC
				(%)			meq 100 g^{-1}		(%)		mg kg-1			$\mu S\ cm^{-1}$
		Graywater	38	32	30	Clay loam	Nd	Nd	Nd	Nd	Nd	18.4	Nd	1900
		Freshwater	64	18	18	Sandy loam	Nd	Nd	Nd	Nd	Nd	1.2	Nd	400
	30-100	Graywater	50	22	28	Sandy clay loam	Nd	Nd	Nd	Nd	Nd	8.4	Nd	1400
		Freshwater	60	24	16	Sandy loam	16	2.5	1.5	0.16	4.6	8.3	7.7	600
	0-15	Graywater	54	32	14	Sandy loam	17	3.6	2.6	0.2	5.6	16	7.4	700
		Freshwater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	5.4	7.8	400
	15-30	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	9.7	7.5	800
		Freshwater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	3.8	7.8	700
Jun ‘10	30-100	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	4.9	7.9	800
		Freshwater	53	31	16	Sandy Loam	17.1	3.2	2.476	0.21	2.5	21.8	7.6	900
	0-15	Graywater	66	17	17	Sandy Loam	18	4.2	3.297	0.31	5.7	108.3	7	1800
		Freshwater	36	36	28	Clay Loam	Nd	Nd	Nd	Nd	Nd	12	7.85	810
	15-30	Graywater	36	34	30	Clay Loam	Nd	Nd	Nd	Nd	Nd	4.2	7.5	500
		Freshwater	40	32	28	Clay Loam	Nd	Nd	Nd	Nd	Nd	0.9	7.8	300
Mar ‘11	30-100	Graywater	40	28	32	Clay Loam	Nd	Nd	Nd	Nd	Nd	0.1	8.07	720
		Freshwater	58	26	16	Sandy Loam	17	4.1	2.982	0.28	2.4	17.1	7.7	900
	0-15	Graywater	64	18	18	Sandy Loam	16.9	5.1	2.871	0.25	2.2	22.8	7.5	1100
		Freshwater	42	26	32	Clay Loam	Nd	Nd	Nd	Nd	Nd	1.2	7.85	510
	15-30	Graywater	42	34	24	Loam	Nd	Nd	Nd	Nd	Nd	1.3	7.62	570
		Freshwater	34	34	32	Clay Loam	Nd	Nd	Nd	Nd	Nd	0.5	8.09	830
Jun ‘11	30-100	Graywater	38	34	28	Clay Loam	Nd	Nd	Nd	Nd	Nd	1	7.8	500

Table C-5. Soil Physical and Chemical Properties at the AZ Household with a New Graywater Irrigation System. (Nd: not determined; continued)

Date	Depth	Irrigation Type	SAR	B	P	K	Zn	Fe	Mn	Cu
						mg kg-1				
		Freshwater	3.7	37	107	390	18.2	223	165	5.8
Oct '08	0-15	Graywater	4.5	37	234	504	19.9	170	145	4.7
		Freshwater	3.7	0.03	16	313	4.6	5.9	6.8	4.1
	0-15	Graywater	3.9	0.03	27	311	8.9	15.2	11	5.5
		Freshwater	3.6	0.01	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Graywater	3.4	0.02	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	3.8	0.01	Nd	Nd	Nd	Nd	Nd	Nd
Jun '09	30-100	Graywater	3.3	0.02	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	3.6	3.1	7.8	252	5.5	6.8	2.6	3.8
	0-15	Graywater	3.4	3.4	7.2	355	7.9	11.3	5.7	11
		Freshwater	4.3	1.3	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Graywater	5.3	2.9	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	5.4	0.8	Nd	Nd	Nd	Nd	Nd	Nd
Jan '10	30-100	Graywater	5.2	1.6	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	3.1	6.7	106	329	4.4	32	131	6.7
	0-15	Graywater	2.4	7.5	148	391	28	43	115	7.5
		Freshwater	3.5	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Graywater	5.8	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Jun '10	30-100	Freshwater	3.9	Nd	Nd	Nd	Nd	Nd	Nd	Nd

Date	Depth	Irrigation Type	SAR	B	P	K	Zn	Fe	Mn	Cu
							mg kg-1			
		Graywater	6	Nd	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	1.6	0.51	87	319	3.79	35.6	101	4.4
	0-15	Graywater	1.6	0.39	92	355	75.4	113	64	8.8
		Freshwater	2.6	0.39	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Graywater	1.7	0.33	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	5.7	0.29	Nd	Nd	Nd	Nd	Nd	Nd
Mar ‘11	30-100	Graywater	1.8	0.21	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	2.8	1	131	355	5.75	36.1	105	8.4
	0-15	Graywater	2.7	0.47	112	313	34	68.7	77	8.6
		Freshwater	3.2	0.33	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Graywater	3	0.4	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	6	0.69	Nd	Nd	Nd	Nd	Nd	Nd
Jun ‘11	30-100	Graywater	2.5	0.26	Nd	Nd	Nd	Nd	Nd	Nd

Table C-6. Soil Physical and Chemical Properties at the CA Household with a New Graywater Irrigation System. (Nd: not determined)

Date	Depth	Irrigation Type	Sand	Silt	Clay	Texture	CEC	Organic Matter	Total C	Total N	NH4-N	NO3-N	pH	EC
				(%)			meq 100 g^{-1}		(%)		mg kg-1			$\mu S\ cm^{-1}$
Sep '08		Freshwater	51	28	21	Loam	27	1.1	0.8	0.1	3.1	1.7	6.3	400
	0-15	Graywater	43	31	26	Loam	23.7	4.8	3.1	0.24	7	2.5	6.2	600
Oct '10	0-15	Freshwater	40	35	25	Loam	15	3.7	2	0.18	2.9	12	5.8	300
	0-15, 2	Graywater	39	30	31	Clay loam	20.2	2.2	1.4	0.15	1.4	3.9	6.2	400
	0-15, 8		41	27	32	Clay loam	20.5	2	1.4	0.13	1.8	4.4	6.6	500
	0-15, 15		41	35	24	Loam	19.6	4.6	2.6	0.23	3.2	5.3	6.3	700
	15-30	Freshwater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	2.9	5.6	200
	15-30, 2	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	5.3	6.6	400
	15-30, 8		Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	3.7	6.2	200
	15-30, 15		Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	7.2	6.4	500
May '11	0-15	Freshwater	41	29	30	Clay Loam	16.7	5.1	2.508	0.2319	2.2	22.8	7.5	1100
	0-15, 2	Graywater	32	33	35	Clay Loam	20.1	4.3	2.14	0.196	3.4	7.1	5.8	400
	0-15, 8		Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	0-15, 15		Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Freshwater	16	34	50	Clay	Nd	Nd	Nd	Nd	Nd	0.3	7.1	312
	15-30, 2	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	0.39	6.2	130
	15-30, 8		20	32	48	Clay	Nd	Nd	Nd	Nd	Nd	0.32	6.3	190
	15-30, 15		14	40	46	Clay	Nd	Nd	Nd	Nd	Nd	0.24	6.3	190

Date	Depth	Irrigation Type	Sand	Silt	Clay	Texture	CEC	Organic Matter	Total C	Total N	NH4-N	NO3-N	pH	EC
				(%)			meq 100 g^{-1}		(%)		mg kg-1			µS cm^{-1}
Oct '11	0-15	Freshwater	20	44	36	Silty Clay Loam	15.4	3.9	2.068	0.1996	2.6	12.4	5.8	400
	0-15, 2	Graywater	14	42	44	Silty Clay	18.5	3.8	1.897	0.1749	1.3	1.8	6.6	400
	0-15, 8		16	40	44	Clay	22.1	3.2	1.793	0.1655	0.88	10.9	6.4	380
	0-15, 15		34	32	34	Clay Loam	16.5	4.3	2.314	0.217	2.8	4.5	7.2	530
	15-30	Freshwater	20	28	52	Clay	ND	Nd	Nd	Nd	Nd	1.2	7	410
	15-30, 2	Graywater	20	32	48	Clay	ND	Nd	Nd	Nd	Nd	3	6.4	380
	15-30, 8		20	32	48	Clay	ND	Nd	Nd	Nd	Nd	6.1	7.2	530
	15-30, 15		16	32	52	Clay	ND	Nd	Nd	Nd	Nd	1.9	7.1	310

Table C-6. Soil Physical and Chemical Properties at the CA Household with a New Graywater Irrigation System. (Nd: not determined; continued)

Date	Depth	Irrigation Type	SAR	B	P	K	Zn	Fe	Mn	Cu
							mg kg-1			
Sep '08	0-15	Freshwater	0.5	0.7	30	419	3.6	242	134	3.7
		Graywater	0.4	0.3	247	662	36	<0.01	252	7.5
Oct '10	0-15	Freshwater	0.5	3	189	278	55	286	35	3
	0-15, 2	Graywater	0.7	4.3	179	367	1.9	277	63	4.3
	0-15, 8		0.5	4.2	159	569	1.8	238	66	4.2
	0-15, 15		0.3	4.9	219	661	27	315	24	4.9
	15-30	Freshwater	1.6		Nd	Nd	Nd	Nd	Nd	Nd
	15-30, 2	Graywater	1.5		Nd	Nd	Nd	Nd	Nd	Nd
	15-30, 8		0.6		Nd	Nd	Nd	Nd	Nd	Nd

Date	Depth	Irrigation Type	SAR	B	P	K	Zn	Fe	Mn	Cu
							mg kg-1			
	15-30, 15		0.5		Nd	Nd	Nd	Nd	Nd	Nd
May '11	0-15	Freshwater		0.47	112	313	34	68.7	77.15	8.637
	0-15, 2	Graywater		0.15	105	324	47.2	200	40.3	4.393
	0-15, 8			Nd	Nd	Nd	Nd	Nd	Nd	Nd
	0-15, 15			Nd	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Freshwater	0.73	0.19	Nd	Nd	Nd	Nd	Nd	Nd
	15-30, 2	Graywater	0.63	0.21	Nd	Nd	Nd	Nd	Nd	Nd
	15-30, 8		0.59	0.2	Nd	Nd	Nd	Nd	Nd	Nd
	15-30, 15		0.53	0.23	Nd	Nd	Nd	Nd	Nd	Nd
Oct '11	0-15	Freshwater	0.4	1.114	108	389	11	191	34.86	3.143
	0-15, 2	Graywater	0.34	0.8587	110	378	12.7	172	25.87	3.647
	0-15, 8		0.45	0.7114	171	394	16.8	222	41.51	4.234
	0-15, 15		0.27	0.521	174	418	25.8	251	23.35	3.172
	15-30	Freshwater	0.33	0.4134	ND	ND	ND	ND	ND	ND
	15-30, 2	Graywater	0.59	0.485	Nd	Nd	Nd	Nd	Nd	Nd
	15-30, 8		0.45	0.2841	Nd	Nd	Nd	Nd	Nd	Nd
	15-30, 15		0.32	1.311	Nd	Nd	Nd	Nd	Nd	Nd

Table C-7. Soil Physical and Chemical Properties at the CO Household with a New Graywater Irrigation System. (Nd: not determined)

Date	Depth	Irrigation Type	Sand	Silt	Clay	Texture	CEC	Organic Matter	Total C	Total N	NH4-N	NO3-N	pH	EC
				(%)			meq 100 g^{-1}		(%)		mg kg-1			$\mu S\ cm^{-1}$
		Freshwater	50	27	23	Sandy clay Loam	19.4	2.9	1.9	0.22	9.2	4.9	7.4	500
Sep '09	0-15	Graywater	64	20	16	Sandy loam	20	5.2	3.8	0.41	6.3	16.4	7	600
		Freshwater	60	19	21	Sandy clay loam	19.5	2.6	2.2	0.2	4.4	8.1	7.7	300
	0-15	Graywater	61	18	21	Sandy clay loam	20.7	4.3	2.9	0.25	5.5	21	7.4	500
		Freshwater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	7.6	7.7	400
	15-30	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	5.3	7.7	300
		Freshwater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	5.9	7.8	300
Jul '10	30-100	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	5	7.9	300
		Freshwater	57	23	20	Sandy clay loam	20.9	4.3	3.2	0.25	2.3	11	7.6	800
	0-15	Graywater	58	22	20	Sandy clay loam	22.2	7.4	3.7	0.28	5.9	51	7	800
		Freshwater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	6.1	7.6	300
	15-30	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	10.7	7.4	500
		Freshwater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	4.1	7.9	300
Sep '10	30-100	Graywater	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	5.4	7.8	300
		Freshwater	50	21	29	Sandy Clay Loam	20.8	2.7	2.03	0.19	3.2	6.1	7.8	400
	0-15	Graywater	46	31	23	Loam	23.4	5.8	3.96	0.32	4	17.8	7.3	600
		Freshwater	20	32	48	Clay	Nd	Nd	Nd	Nd	Nd	4.4	7.9	300
	15-30	Graywater	28	28	44	Clay	Nd	Nd	Nd	Nd	Nd	2.1	7.7	300
Jul '11	30-	Freshwater	12	38	50	Clay	Nd	Nd	Nd	Nd	Nd	1	8	200

Date	Depth	Irrigation Type	Sand	Silt	Clay	Texture	CEC	Organic Matter	Total C	Total N	NH4-N	NO3-N	pH	EC
			(%)				meq 100 g^{-1}		(%)		mg kg-1			$\mu S\ cm^{-1}$
	100	Graywater	10	30	60	Clay	Nd	Nd	Nd	Nd	Nd	1.2	8.1	300
		Freshwater	52	12	36	Sandy Clay	20.2	3.3	2.04	0.202	2.2	1.8	7.3	530
	0-15	Graywater	36	26	38	Clay Loam	21.9	5.1	2.89	0.251	0.19	30.8	7.5	500
		Freshwater	12	36	52	Clay	Nd	Nd	Nd	Nd	Nd	1.7	8.1	300
	15-30	Graywater	18	32	50	Clay	Nd	Nd	Nd	Nd	Nd	12.6	7.7	400
		Freshwater	2	44	54	Silty Clay	Nd	Nd	Nd	Nd	Nd	1.2	7.9	300
Oct '11	30-100	Graywater	10	34	56	Clay	Nd	Nd	Nd	Nd	Nd	15.9	7.9	400

Table C-7. Soil Physical and Chemical Properties at the CO Household with a New Graywater Irrigation System. (Nd: not determined; continued)

Date	Depth	Irrigation Type	SAR	B	P	K	Zn	Fe	Mn	Cu
							mg kg-1			
		Freshwater	<0.1	1.7	4	251	3.9	19.7	8.1	5.3
Sep '09	0-15	Graywater	<0.1	2.6	4.7	349	14.4	24.1	5.9	3.1
		Freshwater	0.3	5.1	23	326	23	93	61	5.1
	0-15	Graywater	0.4	4.1	61	410	22	60	74	4.1
		Freshwater	0.6	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Graywater	1.1	Nd	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	0.7	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Jul '10	30-100	Graywater	1.1	Nd	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	1.4	4.8	100	573	13	61	63	4.9
Sep '10	0-15	Graywater	0.3	5	177	361	26	92	70	5

Date	Depth	Irrigation Type	SAR	B	P	K	Zn	Fe	Mn	Cu
							mg kg-1			
		Freshwater	0.4	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	15-30	Graywater	0.5	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	30-100	Freshwater	0.7	Nd	Nd	Nd	Nd	Nd	Nd	Nd
		Graywater	0.8	Nd	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	0.2	0.15	17	187	4.77	54.8	50	8.8
	0-15	Graywater	0.2	0.53	118	449	31.5	80.6	57	3.7
	15-30	Freshwater	0.3	0.09	Nd	Nd	Nd	Nd	Nd	Nd
		Graywater	0.3	0.19	Nd	Nd	Nd	Nd	Nd	Nd
	30-100	Freshwater	0.4	0.11	Nd	Nd	Nd	Nd	Nd	Nd
Jul '11		Graywater	0.7	0.11	Nd	Nd	Nd	Nd	Nd	Nd
		Freshwater	0.2	0.53	19	356	7.7	58.1	55	3.5
	0-15	Graywater	0.3	0.7	168	347	23	70.6	41	3.5
	15-30	Freshwater	0.3	0.41	Nd	Nd	Nd	Nd	Nd	Nd
		Graywater	0.4	0.56	Nd	Nd	Nd	Nd	Nd	Nd
	30-100	Freshwater	0.3	0.45	Nd	Nd	Nd	Nd	Nd	Nd
Oct '11		Graywater	0.4	0.48	Nd	Nd	Nd	Nd	Nd	Nd

APPENDIX D

INDICATOR ORGANISMS

Table D-1. Total Coliform and Fecal Indicator Counts from Soils (sampled to various depths) Receiving Freshwater or Graywater at Households with Existing Graywater Systems.

Year	State	Treatment	Depth	Total coliforms	*E. coli*	Enterococci	*Clostridium perfringens*
				----------MPN g^{-1} soil----------			CFU g^{-1} soil
2009	AZ	Fresh	0-15 cm	533,000	<1	75,105	<10
		Graywater (recent site)	0-15 cm	62,323	3	699	<10
		Graywater (historic site)	0-15 cm (0-2')	17,195	<1	2,091	<10
			0-15 cm (2-3')	2,668	<1	1,065	<10
			0-15 cm (3-4')	131	<1	747	<10
2008	CA	Fresh	0-15 cm	69,574	<1	150	<10
		Graywater	0-15 cm	82,884	<1	803	<10
2009	CO	Fresh	0-15 cm	14,528	1	366	<10
			15-30 cm	3,462	<1	50	<10
			30-10 cm	216	<1	24	<10
		Graywater	0-15 cm	28,297	1	94	<10
			15-30cm	4,434	<1	50	<10
			30-100cm	958	<1	<1	<10
2010	CO	Fresh	0-15 cm	19	<1	86	Nd
			15-30 cm	38	<1	62	Nd
			30-100cm	156	<1	74	Nd
		Graywater	0-15 cm	1604	1	63	Nd
			15-30 cm	6	<1	11	Nd
			30-100 cm	2005	<1	23	Nd
2011	CO	Fresh	0-15 cm	1,615	<1	30	Nd
			15-30 cm	274	<1	18	Nd
			30-100cm	23	<1	11	Nd
		Graywater	0-15 cm	55,900	1	43	Nd
			15-30 cm	4,016	<1	18	Nd
			30-100 cm	14,944	<1	12	Nd

2008	TX	Fresh	0-15 cm	5,640	136	14,000	375
			15-30 cm	8,500	43	850	<10
			30-100 cm	23,200	216	546	<10
		Graywater	0-15 cm	137,000	543	31,000	<10
			15-30 cm	17,600	160	1,220	<10
			30-100 cm	28,300	1,093	2,230	<10
2009	TX	Fresh	0-15 cm	56,458	254	7,768	Nd
			15-30 cm	177	8	6,683	Nd
			30-60 cm	1,036	36	943	Nd
			60-90 cm	1,239	75	170	Nd
		Graywater	0-15 cm	172,670	65	4,764	Nd
			15-30 cm	>330,033	18	8,850	Nd
			30-60 cm	11,659	<1	1,739	Nd
			60-90 cm	612	<1	65	Nd

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

Table D-2. Total Coliform and Fecal Indicator Counts from Graywater or Soil Irrigated with Freshwater or Graywater at the AZ Household with a New Graywater System.

Year	Water or Soil	Soil Treatment	Soil Depth	Total coliforms	*E. coli*	Enterococci
				----------MPN g^{-1} soil or ml^{-1} water----------		
Oct. 2008	Soil	Fresh	0-15 cm	427,600	6	1,733
		Graywater	0-15 cm	152,600	637	>28,987
June 2009	Graywater		N/A	242,000	502	155,000
	Soil	Fresh	0-15 cm	24,430	<1	2,555
			15-30 cm	2,204	<1	702
			30-10 cm	256	<1	51
		Graywater	0-15 cm	20,663	28	9,639
			15-30cm	175,935	1	2,184
			30-100 cm	24,079	<1	226
Jan. 2010	Graywater		N/A	210	<1	3
	Soil	Fresh	0-15 cm	7,002	1	2,720
			15-30 cm	4,668	1	751
			30-10 cm	633	<1	17
		Graywater	0-15 cm	56,766	10	1,079
			15-30cm	5,158	<1	2,928
			30-100 cm	2,735	<1	235
June 2010	Graywater		N/A	624	1	2
	Soil	Fresh	0-15 cm	36,290	11	35
			15-30 cm	13,180	8	98
			30-100 cm	3,420	<1	152
		Graywater	0-15 cm	160	2	905
			15-30cm	355	2	554
			30-100 cm	9	<1	38
March 2011	Graywater		N/A	>241,960	57,940	53
	Soil	Fresh	0-15 cm	1,249,163	<1	356
			15-30 cm	74	<1	269
			30-100 cm	99	<1	223
	Soil	Graywater	0-15 cm	137,973	133	5,415
			15-30 cm	860	4	1,264
			30-100 cm	226	3	58

June 2011	Graywater		N/A	11	<1	2
	Soil	Fresh	0-15 cm	1,985	5	1,325
			15-30 cm	4,680	<1	4,133
			30-100 cm	420	<1	80
		Graywater	0-15 cm	61,929	93	9,286
			15-30 cm	43,064	61	4,595
			30-100 cm	1,175	4	820

MPN = most probable number, N/A = not applicable

Table D-3. Total Coliform and Fecal Indicator Counts from Graywater or Soil Irrigated with Freshwater or Graywater at the CA Household with a New Graywater System.

Year	Water or Soil	Soil Treatment	Soil Depth	Total coliforms	*E. coli*	Enterococci
				----------MPN g^{-1} soil or ml^{-1} water----------		
Oct. 2008	Soil	Fresh	0-15 cm	31,258	<1	149
		Graywater	0-15 cm	23,369	30	460
Oct. 2010	Graywater		N/A	880	<1	2
	Soil	Fresh	0-15 cm	15,619	<1	446
			15-30 cm	4,523	<1	130
		Graywater, 2' from leachfield	0-15 cm	1,057	<1	1,781
			15-30cm	485	<1	237
		Graywater, 8' from leachfield	0-15 cm	887	<1	476
			15-30cm	3,488	<1	25
May 2011	Graywater		N/A	909	<1	<1
	Soil	Fresh	0-15 cm	213	<1	150
			15-30 cm	15	<1	25
		Graywater, 2' from leachfield	0-15 cm	4,319	<1	743
			15-30 cm	65	<1	75
		Graywater, 8' from leachfield	0-15 cm	24,982	<1	214
			15-30 cm	60	<1	61
Oct. 2011	Graywater		N/A	<1	<1	<1
	Soil	Fresh	0-15 cm	1,771	<1	910
			15-30 cm	3,902	<1	386
		Graywater, 2' from leachfield	0-15 cm	1,476	112	5,989
			15-30 cm	16	<1	1,234
		Graywater, 8' from leachfield	0-15 cm	3,579	<1	7,410
			15-30 cm	39	<1	551

MPN = most probable number, N/A = not applicable

Table D-4. Total Coliform and Fecal Indicator Counts from Graywater or Soil Irrigated with Freshwater or Graywater at the CO Household with a New Graywater System.

Year	Water or Soil	Soil Treatment	Soil Depth	Total coliforms	*E. coli*	Enterococci
				----------MPN g^{-1} soil or ml^{-1} water----------		
Sep. 2009	Soil	Fresh	0-15 cm	1,401	43	1,463
		Graywater	0-15 cm	680,540	51	10,221
July 2010	Graywater		N/A	1,986,000	112,130	<1
	Soil	Fresh	0-15 cm	10,544	<1	433
			15-30 cm	69	<1	247
			30-10 cm	24	<1	12
		Graywater	0-15 cm	1,700	<1	1,496
			15-30cm	25	<1	3,851
			30-100 cm	1	<1	27
Sep. 2010	Graywater		N/A	238	2	2
	Soil	Fresh	0-15 cm	26,464	<1	3,417
			15-30 cm	149,402	<1	659
			30-10 cm	2,615	<1	222
		Graywater	0-15 cm	5,475	<1	5,169
			15-30cm	5,625	7	3,694
			30-100 cm	522	23	206
July 2011	Graywater		N/A	12,740	228	1
	Soil	Fresh	0-15 cm	47,794	<1	2,344
			15-30 cm	37	1	838
			30-10 cm	37	<1	115
		Graywater	0-15 cm	13,015	1	4,083
			15-30cm	27,189	661	2,321
			30-100 cm	38,214	236	633
Oct. 2011	Graywater		N/A	5,190	464	11
	Soil	Fresh	0-15 cm	3,288	1	1,019
			15-30 cm	335	<1	104
			30-10 cm	10	<1	42
		Graywater	0-15 cm	781	<1	2,564
			15-30cm	53	<1	4,103
			30-100 cm	19	<1	274

MPN = most probable number, N/A = not applicable