ABSTRACT

Title of Dissertation: WATER REUSE FOR FOOD PRODUCTION IN THE WEST BANK AND ISRAEL: ASSESSING THE EFFICACY OF HOUSEHOLD GREYWATER TREATMENT SYSTEMS, AND CONSUMER PERCEPTIONS OF REUSE APPLICATIONS

Hillary Anne Craddock, Doctor of Philosophy, 2019

Dissertation directed by: Dr. Amy R. Sapkota, Maryland Institute for Applied Environmental Health

Greywater is increasingly reused for agricultural irrigation in the Middle East. However, there is a dearth of data regarding antibiotics, herbicides, and antibiotic-resistant bacteria in household greywater reuse systems. Additionally, there are minimal data assessing consumer perceptions of water reuse practices. To address these gaps, my dissertation aims were to: 1) evaluate the presence of antibiotics and herbicides in greywater and treated effluent; 2) assess the prevalence of antibiotic-resistant *Enterobacteriaceae* in greywater and treated effluent; and 3) explore consumer perceptions of water reuse practices in Israel and the West Bank.

For Aims 1 and 2, household greywater (n=23), treated effluent (n=23) and pond water (n=12) were collected from four farms in the West Bank from October 2017 to June 2018. The presence of antibiotics and herbicides was quantified using

high performance liquid chromatography-tandem mass spectrometry, *E. coli* were enumerated via membrane filtration, and isolates were tested for antimicrobial susceptibility using microbroth dilution. For Aim 3, surveys (n=236) were administered in Eilat, Israel and Bethlehem, West Bank. Statistical analysis included ANOVA, chi-squared, and Fisher's exact tests.

Multiple antibiotics and herbicides were detected in greywater influent. Removal during treatment was variable across compounds. The majority of influent (76.5%) and effluent (70.6%) samples had detectable levels of *E. coli*. Resistance was most commonly observed against ampicillin, trimethoprim-sulfamethoxazole, tetracycline, and cefazolin. Regarding consumer perceptions, >50% of Israeli respondents were willing to serve raw and cooked produce irrigated with reused water. Palestinian respondents were more willing to engage in high-contact uses than Israeli respondents.

The successful completion of this research has advanced knowledge regarding 1) the persistence of chemical and microbiological contaminants in treated household greywater that is used for food crop irrigation; and 2) consumer acceptance of water reuse practices. Farmers in the West Bank and around the world are combating decreasing quality and quantity of water and will increasingly rely on consumers willing to purchase produce irrigated with treated wastewater. Future work must ensure that farmers have access to safe and abundant irrigation water, and that consumers can be confident that they are purchasing safe food.

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by

Hillary Anne Craddock

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Advisory Committee:
Professor Amy R. Sapkota, Chair
Dr. Kristal Jones
Associate Professor Stephanie Lansing
Associate Professor Emmanuel F. Mongodin
Associate Professor Amir Sapkota
Associate Professor Paul Turner

Dedication

This dissertation is dedicated to the generations of strong, barrier-breaking women in my family. They crossed oceans and continents, fought discrimination, fought for education, and fought to be equals. The stories of those that came before me and the encouragement of my sister (Ms. Allison Craddock), my mother (Dr. Nancy Razickas Craddock), and grandmother (Mrs. Nancy Webb Craddock) helped me to dig deep and keep going.

This dissertation is also dedicated to the men who helped raise me to be the woman I am. My grandfather, Mr. Anthony Razickas (ז"ל), instilled in me stubborn determination, aggressive open-mindedness, and the benefits of approaching all situations, no matter how dire, with calm humor. My father, Dr. John Edwin Craddock III, taught me to have boundless curiosity, pursue learning at every opportunity, to be comfortable working with my hands as well as my mind, and to always value the perspective and wisdom of farmers.

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Thank you to my advisor and mentor Dr. Amy R. Sapkota for your guidance, mentorship, meticulous editing, and inspiration. Your support and belief in me gave me the opportunity to engage in a life-changing research experience. Thank you to my advisor Dr. Paul Turner for your support, advice, and sense of humor. Thank you to the members of my comprehensive exam and dissertation committees for your insightful comments and support. Thank you to my Israeli advisor Dr. Clive Lipchin for your enthusiasm and support.

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Thank you to the staff and faculty of the Arava Institute for Environmental Studies and to the members Kibbutz Ketura for hosting me in Israel and providing a warm, supportive environment for me to grow and thrive professionally and personally. Thank you to the many, many Palestinians, Israelis, and Jordanians who made me feel at home when I was a stranger in a strange land. Thank you to my project co-lead Younes, without whom the work wouldn't have been able to happen, and it wouldn't have been nearly as fun even if it had. Thank you to my awesome intern team in Israel and the West Bank: David, Jake, Sarah, and Carly. Thank you Zubaida and Suleiman for being my Middle East PhD student support network.

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List of Abbreviations

ALA: Alachlor

AMI: Amikacin

AMP: Ampicillin

AS2: Ampicillin/sulbactam

ATR: Atrazine

AXO: Ceftriaxone

AZI: Azithromycin

AZT: Aztreonam

CEC: Contaminant of emerging concern

CIP: Ciprofloxacin

CLSI: Clinical Laboratory Standards Institute

DO: Dissolved oxygen

DOR: Doripenem

EC: Electrical conductivity

ERT: Ertapenem

ERY: Erythromycin

FAZ: Cefazolin

FEP: Cefepime

GEN: Gentamicin

HLB: Hydrophilic lipophilic balance

IL: Israel

IMI: Imipenem

LC/MS-MS: High performance liquid chromatography tandem mass spectrometry

LIN: Linezolid

LEVO: Levofloxacin

MDR: Multidrug-resistant

MERO: Meropenem

MIC: Minimum inhibitory concentration

MIN: Minocycline

MRSA: Methicillin-resistant *Staphylococcus aureus*

NIT: Nitrofurantoin

ORP: Oxidative reduction potential

OXA: Oxacillin

OXO: Oxolinic acid

PA: Palestinian

PEN: Penicillin G

PIP: Piperacillin

PCR: Polymerase Chain Reaction

PT4: Piperacillin/tazobactam

QA/QC: Quality assurance/Quality control

SUL: Sulfamethoxazole

SXT: Trimethoprim/sulfamethoxazole

TAZ: Ceftazidimine

TCC: Triclocarban

TET: Tetracycline

TGC: Tigecycline

TIM2: Ticarcillin/clauvulanic acid

TOB: Tobracycline

TRI: Triflualin

US: United States

UV: Ultra violet

VAN: Vancomycin

VRE: Vancomycin-resistant *Enterococci*

WWTP: Wastewater treatment plant

Chapter 1: Introduction

Groundwater sources around the world, and specifically in the Middle East, are rapidly being depleted and degraded, and groundwater in Israel and the Palestinian Territories has already exhibited signs of overdraw and degradation (Anayah & Almasri, 2009; Avisar, Levin, & Gozlan, 2009; Ghanem, Samhan, Carlier, & Ali, 2011; Jabal, Abustan, Rozaimy, & Najar, 2015; Tal, 2007). Additionally, wastewater mismanagement in the region can negatively impact groundwater, surface water, and agricultural lands (Bieler, 2016; Ezery, 2016; Ghanem & Ahmad, 2015). Agricultural irrigation is a major draw on groundwater sources, and wastewater reuse in agricultural settings has emerged as a potential solution to reduce continual strains on finite groundwater sources as well as to reduce the environmental burden of wastewater mismanagement (Konikow & Kendy, 2005; McIlwaine & Redwood, 2011; Sapkota, 2019; Shomar & Dare, 2015).

However, there are downsides to reusing treated wastewater for irrigation. Treated wastewater may still contain a number of microbiological and chemical constituents that could be harmful to the environment, food safety, and public health (Carey et al., 2016; Kulkarni et al., 2018; Malchi, Maor, Tadmor, Shenker, & Chefetz, 2014; Paltiel et al., 2016; Tal, 2016). Previous studies investigating multiple types of wastewater, including treated water being used for irrigation, have identified *Escherichia coli*, *Salmonella enterica*, *Shigella* sp., *Klebsiella pneumoniae, Legionella* sp., *Enterococci*, and *Staphylococcus aureus* in these water sources (Benami, Gillor, & Gross, 2016; Carey et al., 2016; Goldstein et al., 2012; Gross, Kaplan, & Baker, 2007; Kulkarni et al., 2018; Maimon, Friedler, & Gross, 2014; Ronen, Guerrero, & Gross, 2010; Rosenberg Goldstein et al., 2014; Troiano, Beneduce, Gross, & Ronen, 2018). In a subset of these studies antibiotic resistance, including multidrug-resistance, was observed (Carey et al., 2016; Rosenberg Goldstein et al., 2014; Troiano et al., 2018), thus indicating that wastewater reuse could potentially contribute to the selection of antibiotic-resistance in the environment and increasing rates of resistant infections. Water contaminants of emerging concern, such as antibiotics critical to human medicine, herbicides, and chemicals originating from personal care products, have also been noted in wastewater sources in the US, Israel, and Saudi Arabia (Alidina et al., 2014; Batt, Kim, & Aga, 2007; Eriksson, Auffarth, Eilersen, Henze, & Ledin, 2003; Kulkarni et al., 2017). Furthermore, Israeli researchers have observed that produce irrigated with treated wastewater can introduce measurable levels of pharmaceuticals into the food supply (Malchi et al., 2014; Paltiel et al., 2016). Israel has been using treated wastewater for decades, and as such its challenges and successes regarding wastewater reuse for agricultural irrigation are potentially applicable to urban and suburban regions in developed countries around the world, including the US.

While small-scale greywater treatment systems have emerged as a potential solution to agricultural water scarcity in developing countries, little has been done to investigate the presence of antibiotics and herbicides in greywater and evaluate whether these systems are effective at removing these contaminants (Chen, Wei, et al., 2016; Eriksson et al., 2003; Zedek et al., 2015). Additionally, research regarding bacterial contamination of greywater has generally focused on the presence and enumeration of fecal indicator bacteria, and research on the presence of antibiotic-resistant bacteria in greywater systems is limited (Al-Gheethi, Noman, & Ismail, 2015; Benami et al., 2016; Troiano et al., 2018). These knowledge gaps must be addressed to fully assess the potential food safety risks stemming from off-grid greywater reuse. These findings regarding off-grid greywater reuse systems can be applicable to both developing scenarios around the world as well as off-grid, remote settings in developed countries like the US.

Furthermore, while some preliminary studies have investigated the public's willingness to accept the use of reused wastewater and other non-groundwater irrigation water sources on food crops, studies in the Middle East are limited and, to our knowledge, no published studies have explored international comparisons regarding consumer's response to agricultural water reuse (Drechsel, Mahjoub, & Keraita, 2015; Friedler, 2008). Understanding community knowledge levels and concerns and responding with appropriate outreach and engagement can be important for new wastewater reuse endeavors (Dolnicar, Hurlimann, & Nghiem, 2010). This is especially critical when assessing consumer willingness to reuse water in Israel and the West Bank, as there is a significant amount of transboundary trade in agricultural products between the two (Bank of Israel, 2014). Moreover, this is a comparison, and relationship, that could be of notable importance to the US as well. For example, frequent US agricultural trading partners may want to utilize wastewater reuse in a developing scenario (i.e. Mexico), and purchasing nations (i.e. Canada, the European Union) may not accept treated wastewater-irrigated produce (Bastian & Murray, 2012; USDA ERS, 2017).

My dissertation research sought to fill these knowledge gaps by generating data that addressed the following three research aims:

Aim 1: To investigate the presence and concentration of antibiotics and herbicides in the influent and effluent of two types of small-scale, off-grid greywater treatment systems (upflow gravity filtration and constructed wetlands) in the West Bank over time.

Aim 2: To quantify *E. coli* levels as well as describe the presence of phenotypic antibiotic resistance among *Enterobacteriaceae* isolates recovered from the influent and effluent of two types of small-scale, off-grid greywater treatment systems (upflow gravity filtration and constructed wetlands) in the West Bank over time.

Aim 3: To evaluate knowledge and acceptance of food crops grown with recycled water among two populations: a majority Israeli Jewish population in Eilat, Israel and a majority Palestinian Muslim population in Bethlehem, West Bank.

The dissertation is organized in the following fashion:

Chapter 2: Background

This chapter provides background information on the issues of water scarcity and security in Israel and the Palestinian Territories, wastewater reuse globally and in Israel and the Palestinian Territories, and chemical and microbial contaminants in wastewater. In addition, the chapter provides relevant information that would inform the monitoring and regulation of these contaminants in wastewater in the West Bank, as well as information on consumer acceptance of wastewater reuse globally, in Israel, and in the West Bank.

Chapter 3: Antibiotic and herbicide concentrations in household greywater reuse systems and pond water used for food crop irrigation: West Bank, Palestinian Territories

This chapter presents the manuscript that resulted from the study conducted to address Aim 1:

Objective: To assess antibiotics and herbicides in household greywater influent as well as their persistence in effluent.

Approach: Household greywater samples (influent and effluent, n=46) and irrigation pond water samples (n=12) were collected from four small farms in the West Bank, Palestinian Territories from October 2017 to June 2018. All samples were analyzed using high performance liquid chromatography tandem mass spectrometry (LC-MS/MS) for the following antibiotics and herbicides: alachlor, ampicillin, atrazine, azithromycin, ciprofloxacin, erythromycin, linezolid, oxacillin, oxolinic acid, penicillin G, sulfamethoxazole, triclocarban, tetracycline, triflualin, and vancomycin. Data were analyzed using descriptive statistics and repeated-measures ANOVA.

Key findings: All tested antibiotics and herbicides were detected in greywater influent samples. When comparing influent to effluent concentrations, removal was observed for azithromycin, alachlor, linezolid, oxacillin, penicillin G, pipemidic acid, sulfamethoxazole, triclocarban, and vancomycin. Removal was not observed for atrazine, ciprofloxacin, erythromycin, oxolinic acid, tetracycline, and trifluralin. Pond water was found to contain the majority of tested contaminants.

This manuscript is in revision with *Science of the Total Environment*.

Chapter 4: Antibiotic-resistant *Escherichia coli* and *Klebsiella* sp. in greywater reuse systems and pond water used for agricultural irrigation in the West Bank, Palestinian Territories

This chapter presents the manuscript that resulted from the work carried out to address Aim 2:

Objective: To assess *E. coli* levels, basic quality parameters, and the presence of antibiotic-resistant bacteria in household greywater as well as treated effluent.

Approach: Greywater influent (n=58), effluent (n=16), and pond water (n=8) samples were collected from four farms in the West Bank between November 2017 and June 2018. Samples were tested using standard methods for *E. coli*, turbidity, pH, electrical conductivity, and dissolved oxygen. Isolates recovered from all samples were analyzed for phenotypic expression of antibiotic resistance using micro-broth dilution.

Key findings: More than half of influent and effluent samples had detectable levels of *E. coli* (76.5% and 70.6% respectively). A greater proportion of effluent isolates were fully susceptible to all tested antibiotics when compared to influent isolates (28.6% vs 18.6%). Across all influent, effluent and pond samples, resistance was most commonly observed against ampicillin (69.3% of all isolates), trimethoprim-sulfamethoxazole (11.4%), tetracycline (9.1%), and cefazolin (7.9%). Among all isolates, 7.9% were multidrug-resistant (MDR).

This manuscript will be submitted to *Environmental Research.*

Chapter 5: Perceptions on the use of recycled water for produce irrigation and household tasks: A comparison between Israeli and Palestinian consumers

This chapter presents the manuscript that resulted from the study carried out to address Aim 3:

Objective: To assess consumer willingness regarding reused wastewater for multiple applications in Israel and the West Bank.

Approach: 127 Israelis and 109 Palestinians were surveyed, for a total of 236 survey respondents. Surveys assessed general knowledge about water scarcity issues and water reuse and willingness to serve raw and cooked produce irrigated with recycled water. The survey also assessed willingness to use recycled water to wash clothes, bathe, wash dishes, wash produce, and drink. Descriptive statistics, Chi-squared analysis, and Fisher's exact tests were used to analyze the data.

Key findings: Perceptions of regional water scarcity and water contamination varied between the two populations; Palestinians were more likely than Israelis to agree that their region had recently experienced drought or water contamination. Israeli willingness to use recycled water for various purposes ranged from 8.3% - 55.1%, and more than half of Israeli respondents were willing to serve both raw and cooked produce irrigated with recycled water. Willingness to use recycled water ranged from 28.9% - 41.5% among the Palestinian respondents, and Palestinian respondents were more willing to engage in high-contact uses (i.e. drinking and cooking) than Israeli respondents.

This manuscript will be submitted to *Desalination*.

Chapter 6: Conclusions, Public Health Implications, and Future Research

Finally, this chapter provides an overview of the findings of this dissertation, specifically through the lens of public health. While focused on Israel and the Palestinian Territories, these findings have implications relevant to the US. The results of these studies have relevance to food safety, food security, the health of farmers and rural people in the West Bank, and the growing global challenge of increasing rates of antibiotic resistance. Additionally, this research has identified future areas of research, namely the potential influence of antibiotic residues on bacterial populations in off-grid wastewater, consumer acceptance of wastewater reuse among diverse populations in Israel, and the overall performance of off-grid wastewater treatment systems.

Chapter 2: Background

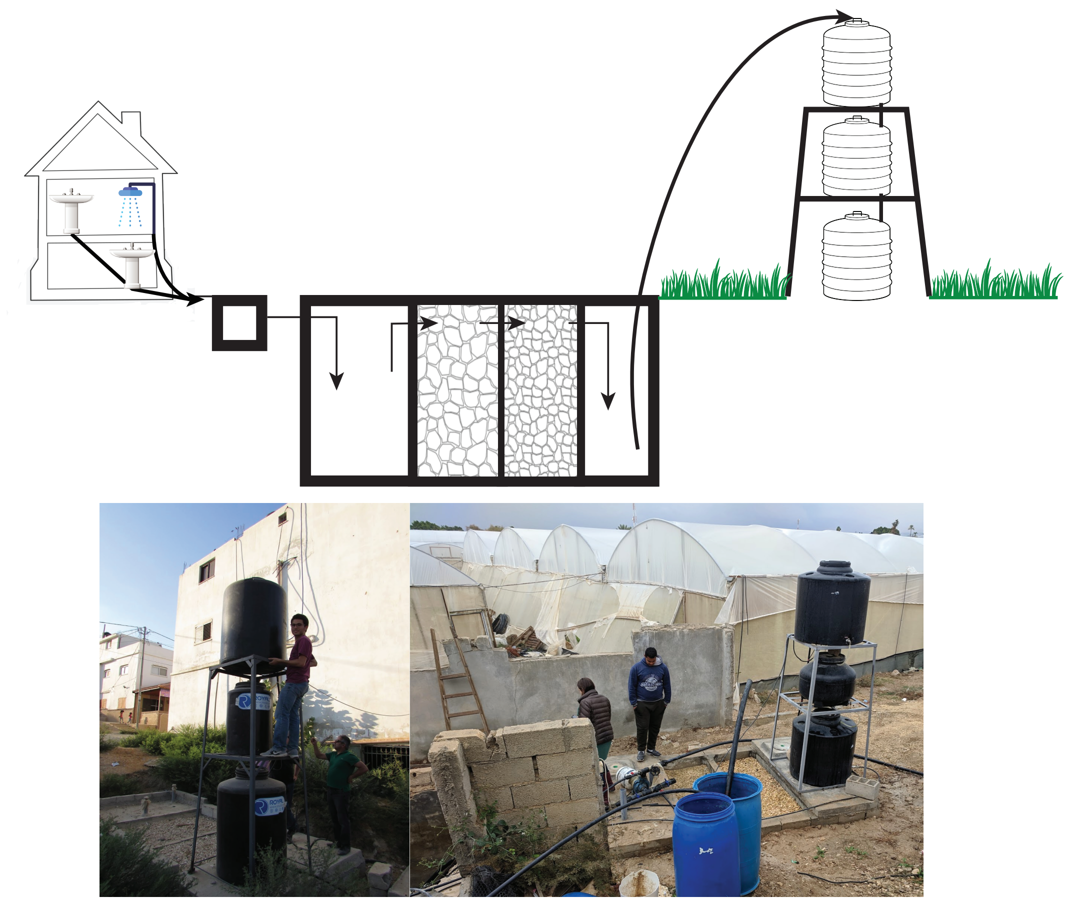
Wastewater Reuse

Groundwater is a heavily-used resource around the world, especially in areas where surface water and rainwater are insufficient to meet the needs of households, industry, and agriculture. This heavy use and sometimes overuse of groundwater has put significant stress on aquifers around the world, and this stress is projected to increase (Gleeson, Wada, Bierkens, & van Beek, 2012). Wastewater treatment and reuse is a potential solution to groundwater overuse, especially for agricultural irrigation.

There are two main types of municipal wastewater. Greywater is defined as all wastewater that does not include sewage (or blackwater), and thus can include water from kitchen sinks, hand washing basins, laundry basins or machines, and bath tubs or showers. Greywater is a frequent target for reuse as it is considered to be lower in bacteria and chemical pollutants than blackwater-containing wastewater (Donner et al., 2010; Gross et al., 2007). However, kitchen sink water is often classified as "dark greywater" and is frequently excluded from greywater reuse projects due to high bacterial content (Gross, Kaplan, & Baker, 2007). The second main type of municipal wastewater is water that often originates from wastewater treatment plants (WWTPs) and includes both greywater and blackwater.

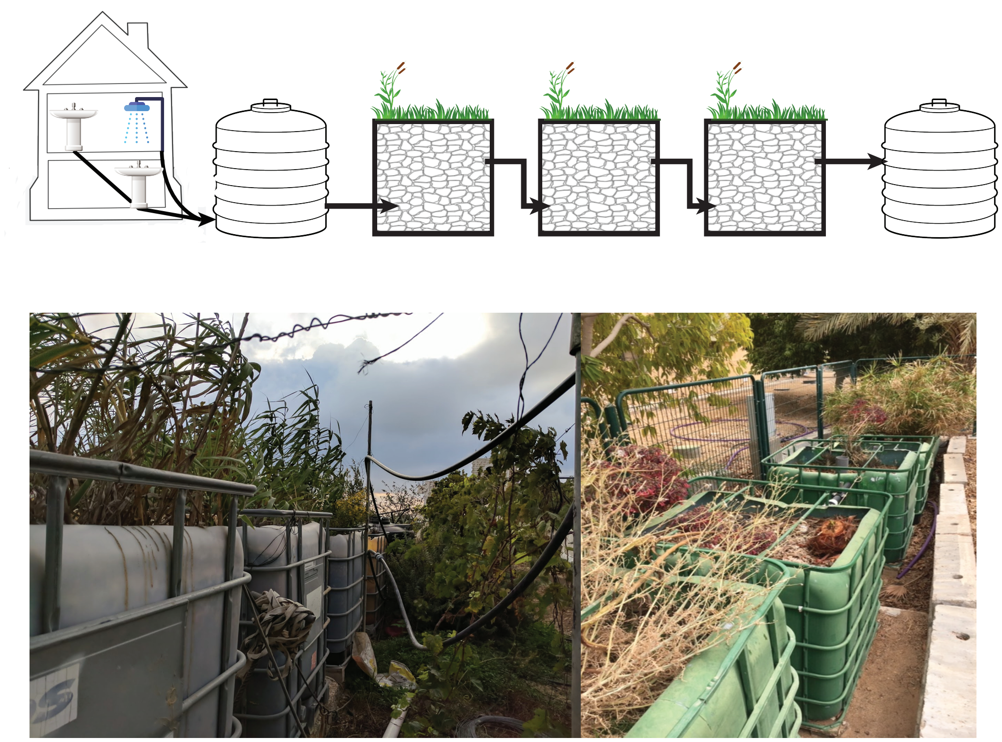
Municipal wastewater treatment is classified into primary, secondary, and tertiary levels. Primary treatment is the removal of large objects (i.e. branches, leaves, and trash) from the wastewater. Secondary treatment consists of biological treatment; the main purpose of this stage is to degrade biological material and reduce the pathogen load (Maier, Pepper, & Gerba, 2009). Tertiary treatment is an additional step that “polishes” the effluent for release into the environment or reuse, and can include chlorination, sand filtration, ultraviolet (UV) treatment, reverse osmosis, and coagulation (Maier, Pepper, & Gerba, 2009). Tertiary treatment is commonly employed in wastewater treatment plants (WWTPs) in developed nations, including Israel and the US (Dotan et al., 2016; Maier et al., 2009). Two popular modalities for greywater treatment and reuse, upflow gravel filtration systems (Figure 1) and constructed wetlands (Figure 2), utilize secondary treatment approaches.

Figure 1: Schematic of upflow gravel greywater treatment system (top), and photos of upflow gravel greywater treatment systems in the West Bank, Palestinian Territories (bottom).



Constructed wetlands are typically a series of containers filled with gravel and plants. Breakdown of contaminants is achieved through exposure to biofilms, UV exposure, and also via uptake by the plants (phytoremediation) (Little, 2014). Upflow gravel filtration systems combine passive gravel filtration with active pumping of wastewater through anaerobic tanks, and breakdown is achieved through exposure to biofilms (Harman et al., 2017). These off-grid systems, while cheaper and easier to implement in low-resource settings than large-scale WWTPs, often have challenges including bad smell and unacceptably high levels of bacteria (Alfiya, Gross, Sklarz, & Friedler, 2013; Harman et al., 2017; Little, 2014).

Figure 2: Schematic of constructed wetlands greywater treatment system (top), and photos of constructed wetlands systems (bottom) in the West Bank, Palestinian Territories (left) and Ketura, Israel (right).



Research in Israel has also noted that greywater can be high in bacteria, salts, surfactants, and other contaminants, and thus, improperly treated greywater can potentially present a health risk as well as degrade soil quality (Benami et al., 2016; Shafran, Gross, Ronen, Weisbrod, & Adar, 2005). This is especially problematic in the Middle East and other arid regions, as previous studies have found that evapotranspiration that occurs during these treatment methods can concentrate contaminants in greywater (Leas, Dare, & Al-Delaimy, 2014; Shafran et al., 2005).

Both the constructed wetlands and upflow gravel filtration system configurations depicted in Figures 1 and 2 are estimated to have flow rates of approximately 500L/day (S. Halasah, Personal Communication, Jan 26-27, 2019; M. P. Hinde, Personal Communication, Jan 2-27, 2019). Retention time for constructed wetlands is dependent on the number and size of tanks, and it is estimated that for each 1,000L tank there is a retention time of two days (S. Halasah, Personal Communication, Jan 26-27, 2019). The system depicted in Figure 1, for example, has three 1,000L tanks and would thus have a retention time of six days (S. Halasah, Personal Communication, Jan 26-27, 2019; M. P. Hinde, Personal Communication, Jan 2-27, 2019; Little, 2014). Retention time in upflow gravel filtration systems is highly dependent on the dimensions of the various tanks seen in Figure 2; with the dimensions commonly used by aid organizations in the West Bank, retention time is estimated to be 25 days (S. Halasah, Personal Communication, Jan 26-27, 2019; M. P. Hinde, Personal Communication, Jan 2-27, 2019).

Water Scarcity and Wastewater Reuse in Israel and the Palestinian Territories

Water Scarcity and Water Security

The Middle East is a region notorious for its water scarcity, and Israel and the Palestinian Territories are no exception. Farmers are often reliant on groundwater and surface water for irrigation, as monthly precipitation averages range from zero to 100 mm (World Bank Group, 2019a, 2019b). Overuse of freshwater sources has significantly depleted aquifers and surface waters, and overdraw of aquifers has increased the impact of surface pollutants on groundwater quality (Anayah & Almasri, 2009; Jabal et al., 2015). Groundwater contamination with antibiotics via leakage from agricultural sources has already been observed in Israel (Avisar et al., 2009), and low levels of pesticides have been observed in groundwater in the Gaza Strip and the West Bank (Ghanem et al., 2011; Hallaq & Elaish, 2012).

Given the tenuous nature of these groundwater sources, actions should be taken to closely guard against any potential source of pollutant infiltration. Controlled field experiments have shown that contaminants from wastewater originating from households and land use practices can potentially impact groundwater resources (Cordy et al., 2004). Wastewater in the West Bank and in off-grid Israeli Bedouin villages is often collected in unlined cesspits, and this practice puts already-strained groundwater sources at risk for pollution (Bieler, 2016; Ezery, 2016). In the West Bank overall, 60.4% of households report using porous cesspits for their wastewater, and even more households (79.9%) in the southern part of the West Bank utilize porous cesspits (Palestinian Central Bureau of Statistics, 2008).

Wastewater mismanagement also poses a risk to surface water and agricultural lands. Cesspits must be emptied on a regular basis, and after the wastewater is pumped and collected by a private company that company may go on to dump the wastewater in a nearby wadi (valley) (All-Halih, 2008). When raw wastewater is discharged into a local wadi, it can overflow into adjacent agricultural fields. The subsequent introduction of microbial and chemical contaminants to the soil can negatively impact the quality of the land, and surveys of agricultural villages in the West Bank found that farmers have abandoned previously-cultivated land due to regular flooding with wastewater (Ahmad & Ghanem, 2015). A third to a half of those surveyed in the same villages perceived that they had contracted an infectious disease as a result of the wastewater flooding (Ahmad & Ghanem, 2015). The treatment and reuse of wastewater is a potential solution to the problems of environmental pollution and overutilization of groundwater sources in Israel and the Palestinian Territories (Gross et al., 2007; Shaheen, 2003; Shomar & Dare, 2015). Furthermore, these solutions can also address environmental problems in the US. While wastewater mismanagement is not nearly so pronounced in the US, groundwater can be infiltrated by poorly managed wastewater, and groundwater overdraw is also a challenge in several parts of the US (Batt, Snow, & Aga, 2006; Campagnolo et al., 2002; Gleeson, Wada, Bierkens, & van Beek, 2012; Oun, Kumar, Harrigan, Angelakis, & Xagoraraki, 2014).

Wastewater Reuse in Israel

Currently, 86% of wastewater in Israel is treated and reused, and 50% of irrigation water in Israel is from treated effluent (Tal, 2016). In contrast, the US treats 71% of its wastewater, and it is estimated that 4% of treated wastewater is reused (Sato, Qadir, Yamamoto, Endo, & Zahoor, 2013). Israel also encourages the use of treated wastewater for agricultural irrigation by utilizing a pricing structure. When farmers purchase irrigation water, treated wastewater is the cheapest option while pumped groundwater is the most expensive (Marin, Tal, Yeres, & Ringskog, 2017). Many Israelis are connected to a wastewater grid, but some off-grid greywater reuse has been implemented (Benami et al., 2016; Gross et al., 2007; Maimon et al., 2014; Troiano et al., 2018).

Israeli WWTPs use advanced tertiary wastewater treatment to provide high-quality irrigation water (Dotan et al., 2016). While this has allowed for significant growth of Israeli agriculture in an otherwise water-scarce environment, these treatment modalities may be insufficient to completely remove contaminants such as antibiotics and antibiotic-resistant bacteria (Carey et al., 2016; Goldstein et al., 2012; Kulkarni et al., 2018). Previous research in Israel has noted that the increased use of treated effluent in agricultural irrigation contributes to salinization of soil, as well as the introduction of pharmaceutical compounds into the food chain (Malchi et al., 2014; Paltiel et al., 2016; Paz et al., 2016; Tal, 2016). Utilization of desalination technologies such as reverse osmosis as an additional treatment step, as well as soil amendments, have been suggested to address the growing concerns of salinity, pharmaceutical compounds, and pathogens (Tal, 2016).

To address the issues of bacterial and chemical contaminants, Israel drafted the world's first set of wastewater reuse standards in 1953, and wastewater reuse standards are regularly updated (Inbar, 2007; Tal, 2016). The most recent version of the standards covers multiple measures of water quality, including but not limited to turbidity, pH, fecal coliforms, detergents, oils, and heavy metals (Inbar, 2007). However, the standards are lacking any pathogen restrictions beyond coliforms (Inbar, 2007) and have not yet addressed the persistence of pharmaceuticals in reused water.

Wastewater Reuse in the West Bank

A significant gap exists between the water available in the Palestinian Territories, both surface water and groundwater, and the amount of water needed per year (Fatta, Salem, Mountadar, Assobhei, & Loizidou, 2004; Fatta, Skoula, et al., 2004). It is hypothesized that treated wastewater could fill a large proportion of that gap, especially in regards to agricultural irrigation. However, there is a significant disparity in the reuse of treated wastewater when comparing the Palestinian Territories to their Israeli neighbors (Dotan et al., 2016; Fatta, Skoula, et al., 2004; McIlwaine & Redwood, 2011).

In the West Bank, 1-6% of wastewater is collected and reused, and there is only one functioning tertiary-level treatment plant (Dotan et al., 2016; McIlwaine & Redwood, 2011; Mizyed, 2013). Most WWTPs only implement primary or secondary treatment (Dotan et al., 2016; McIlwaine & Redwood, 2011), and lightly- and often improperly-treated wastewater is discharged into nearby wadis (Al-Sa'ed, 2010;

Mizyed, 2013). Additional studies have found that these WWTPs are overloaded and do not have sufficient capacity to treat all of the wastewater produced by the communities in the network (McNeill, Almasri, & Mizyed, 2009).

Moreover, there are multiple bureaucratic and political barriers to large-scale wastewater reuse in the West Bank. Previous studies have found that infrastructure, regulatory oversight, and coordination among government actors within the West Bank is lacking (Al-Khatib, Shoqeir, Özerol, & Majaj, 2017). Most wastewater treatment plant construction efforts in the West Bank would require permission from and cooperation between the Israeli government and the Palestinian Authority (Al-Sa'ed, 2010). However, there is a Palestinian standard for wastewater irrigation reuse in place. It includes standards for a wide variety of contaminants, such as fecal coliforms, *Escherichia coli,* pH, dissolved oxygen, detergents, and heavy metals (Palestine Standards Institute (PSI), 2003). Due to the lack of capacity for large-scale reuse, small-scale, off-grid wastewater treatment solutions have been implemented in the West Bank (McIlwaine & Redwood, 2011). These practices have emerged as a result of both aid efforts and adaptations to the common practice of using untreated greywater for irrigation (Bieler, 2016; Ezery, 2016).

Importantly, Palestinian farmers are generally willing to adapt reused wastewater for irrigation (Dare & Mohtar, 2018; Ghanem, Isayed, & Abu-Madi, 2010; Mizyed, 2013; Shomar & Dare, 2015). Several surveys have been conducted among West Bank farmers, and have generally found that between 47% and 83% would be willing to reuse treated wastewater for irrigation (Al-Kharouf, Al-Khatib, & Shaheen, 2008; Ghanem, 2016; Ghanem et al., 2010). However, a recent survey of farmers in multiple West Bank villages found that they had a low opinion of crops irrigated with treated wastewater (1.9 on a five-point likert scale), and the farmers surveyed also felt that the general public in the West Bank had a similarly low opinion (1.8 on a five-point likert scale) (Dare & Mohtar, 2018). This understanding of both consumer and farmer acceptance is a critical piece of implementing wastewater reuse in the Middle East and around the world (Al-Sa'ed, Mohammed, & Lechner, 2012; A. Dare & Mohtar, 2018; Suri et al., 2019).

Public Acceptance of Wastewater Reuse

Technological advances must be accepted by communities in order to be successful (Baumgartner, Murcott, & Ezzati, 2007). Wastewater reuse in particular is an emotionally charged technological advance, and an understanding of what drives a community's emotional response, as well as what communities know and think about reuse, and which sociodemographic and behavioral factors are influencing acceptance, is critical for its success and utilization (Hartley, 2006; Miller & Buys, 2008; Morgan & Grant-Smith, 2015; Rozin, Haddad, Nemeroff, & Slovic, 2015). Indeed, reuse projects have been scuttled due to poor community acceptance in Australia, the US, and around the world (Hurlimann, 2008; Hurlimann & Dolnicar, 2010; Schwartz, 2015).

United States and Australian Research on Public Acceptance of Wastewater Reuse

Most of the foundational research in wastewater reuse has taken place in the US and Australia, and these studies have found that acceptance of wastewater reuse is affected by a variety of factors including but not limited to wastewater experience, trust in authority, and drought awareness (Hartley, 2006; Miller & Buys, 2008; Morgan & Grant-Smith, 2015; Rozin et al., 2015). Familiarity with wastewater and experience with reusing water is associated with acceptance, for both potable and non-potable reuse (Hartley, 2006). A long-term Australian study of a community that incorporated reused wastewater into its water supply found that as time went on acceptance for all uses of treated wastewater increased in the community, including acceptance of reused water as source water for drinking water treatment (potable reuse) (Hurlimann, 2008). In a recent survey of Mid-Atlantic residents in the US, it was found that prior knowledge of reuse was associated with acceptance of wastewater for agricultural irrigation (Savchenko, Kecinski, Li, & Messer, 2018).

A survey in Australia found that trust in the water authority was strongly tied to acceptance of potable reuse (Ross, Fielding, & Louis, 2014). A survey of California (US) college students found that the majority were willing to accept potable reuse, and the main factors that appeared to be driving acceptance were drinking tap water at home, as well as a strong perception of drought and water shortages in their region (Hummer, 2017).

Surveys in the US and in Australia have both found that acceptance of potable reuse is lower than acceptance for other uses, and these studies also note that low-contact options (i.e. toilet flushing, washing clothes, and irrigation) are more accepted than high-contact options (i.e. bathing, cooking, and drinking) (Dolnicar et al., 2010; DuBose, 2009). When compared to other “non-conventional” water sources, treated wastewater is often considered unfavorable. A drinking water study in Australia found that participants preferred treated rainwater, stormwater, and desalinated water to treated wastewater (Fielding, Gardner, Leviston, & Price, 2015). A study in the Mid-Atlantic region of the US parsed between treated greywater and blackwater for agricultural irrigation and found that treated blackwater was second only to treated industrial water in terms of rejection, and treated greywater was more acceptable than treated brackish water but less acceptable than treated stormwater and rainwater (Savchenko et al., 2018).

Middle Eastern Research on Public Acceptance of Wastewater Reuse

Most research investigating acceptance of wastewater reuse in the Middle East focuses on agricultural irrigation reuse. A survey in Jordan found that water-scarce communities were willing to accept off-grid greywater treatment and reuse (Ghrair, Al-Mashaqbeh, & Megdal, 2015), and a survey carried out concurrently in Tunisia and Jordan found that consumers in both countries were willing to accept crops irrigated with treated wastewater (Abu Madi, Al-Sa’ed, Braadbaart, & Alaerts, 2008). In two studies investigating general reuse acceptance, in Iran and Kuwait respectively, patterns of acceptance similar to those in the US and Australia were observed, with low acceptance for high-contact uses such as cooking and drinking and high acceptance for low-contact uses like irrigation (Alhumoud, Behbehani, & Abdullah, 2003; Baghapour, Shoshtarian, & Djahed, 2016).

Israeli and Palestinian Research on Public Acceptance of Wastewater Reuse

Despite long-term wastewater reuse in Israel, minimal data have been collected regarding the Israeli public's acceptance of wastewater reuse. One 2006 study found no relationship between acceptance and knowledge of water issues, awareness of environmental issues, knowledge of wastewater treatment technologies, or trust in authorities to properly run the systems (Friedler, 2008). However, believing that there were health risks associated with reuse was significantly associated with decreased acceptance. Degree of contact was a significant contributor to acceptance, and acceptance for agricultural recycling again varied based on perceived contact. Vegetable irrigation received 48% acceptance, orchard irrigation received 53% acceptance, and aquifer recharge received 67% acceptance (Friedler, 2008).

A moderate amount of data exists examining the public's acceptance of agricultural crops irrigated with treated wastewater in the West Bank, and the results are varied. A 2001 survey in multiple cities around the West Bank found that 65% of respondents would buy produce irrigated with treated wastewater (Faruqui, Biswas, & Bino, 2000). Surveys conducted in the central region of the West Bank, around Ramallah, found acceptance of treated wastewater reuse in agriculture ranged from 25% - 89.7% (Abu Madi, Mimi, & Abu-Rmeileh, 2008; Al-Sa'ed & Mubarak, 2006). These Ramallah-area surveys also noted that health concerns were a primary barrier to acceptance (Abu Madi, Mimi, et al., 2008; Mahmoud & Mimi, 2008).

In the southern part of the West Bank, a survey in the Jerusalem and Bethlehem Governorates found that 41.9% of consumers would accept produce irrigated with treated wastewater (Ghanem, 2012). In the Hebron area, Ghanem et al (2010) also found that less than half of consumers (32-47%) were willing to accept fruits and vegetables irrigated with treated wastewater. Cooked produce was more accepted by consumers (Ghanem et al., 2010). It is important to note that, regarding the southern part of the West Bank, respondents living or working in Bethlehem are exposed to water shortages on a regular basis. Water shortages and cuts have been occurring regularly around Bethlehem for several years due to shortages in supply as well as ill-maintained infrastructure (Applied Research Institute - Jerusalem, 2008). Even those not directly impacted are made aware of these shortages, as protests frequently occur (Ma'an News, 2016; Nazzal, 2016).

Understanding community knowledge levels and concerns among Israelis and Palestinians together is important, as these two populations engage in a large amount of transboundary trade in agricultural products. Most produce grown in the West Bank is sold in the West Bank, and the remainder is primarily exported to Israel (Bank of Israel, 2014; Venghaus, 2017). The West Bank also imports a large quantity of agricultural products from Israel (Bank of Israel, 2014). Due to these economic factors, understanding how these two populations accept produce irrigated with treated wastewater is critical to ensuring that these products will have a market.

Chemical and Microbial Contaminants in Treated Wastewater

Bacteria and Antibiotic Resistance in Wastewater

It is known that even wastewater treated to the tertiary level can contain pathogens, and furthermore that these pathogens can be resistant to antibiotics.

Research investigating tertiary-treated municipal wastewater, including water being used for irrigation, has identified *Legionella* sp., vancomycin-resistant *Enterococci* (VRE), and methicillin-resistant *Staphylococcus aureus* (MRSA) in both influent and treated effluent samples (Carey et al., 2016; Goldstein et al., 2012; Kulkarni et al., 2018; Rosenberg Goldstein et al., 2014). Additionally, antibiotic resistance genes persisting in treated wastewater can be transmitted among environmental bacteria once effluent is introduced into the environment (Berglund, Fick, & Lindgren, 2015; Martinez, 2009; Rizzo et al., 2013). Pathogens are also observed in treated greywater, and include but are not limited to *E. coli*, *Salmonella enterica*, *Shigella* sp., and *Klebsiella pneumoniae* (Benami et al., 2016; Gross et al., 2007; Maimon et al., 2014; Ronen et al., 2010).

Studies investigating antibiotic resistance in greywater are limited, with one study investigating greywater from a pharmacy school (Al-Gheethi et al., 2015), one study evaluating an open channel receiving greywater from an urban neighborhood (Nuñez, Tornello, Puentes, & Moretton, 2012), and one assessing household greywater in Israel (Troiano et al., 2018). Significant levels of resistance were detected; for example, 34% of Gram-negative bacteria recovered from greywater in an open-ditch disposal system in Buenos Aires, Argentina in Nunez et al. (2012) were resistant to ampicillin.

Bacterial Levels and Antibiotic Resistance in Wastewater in Israel and the West Bank

Two studies from the West Bank found high levels of fecal coliforms in constructed wetland system influent, and even post-treatment bacterial levels were still frequently in violation of Palestinian reuse standards (Arafeh, 2012; Little, 2014). Studies of similar off-grid greywater treatment systems in Israel also found high levels of fecal coliform bacteria (Gross et al., 2007; Ronen et al., 2010). Culture-based and culture-independent methods have been utilized in Israel to identify pathogens in both untreated and treated greywater, and, in addition to *E. coli*, numerous other *Enterobacteriaceae* have been identified, including *S. enterica*, *Shigella* sp., and *K. pneumoniae* (Benami et al., 2016; Gross et al., 2007; Maimon et al., 2014; Ronen et al., 2010). Israeli studies support the general findings that kitchen greywater contributes a large amount of bacteria (Ronen et al., 2010); however, due to low household water use, greywater reuse systems in the West Bank typically include kitchen greywater.

As noted earlier, research regarding antibiotic-resistant bacteria in greywater is lacking, however, one Israeli study identified tetracycline-resistant bacteria in Israeli household greywater, and also noted a statistically significant increase in tetracycline-resistant bacteria in treated greywater as opposed to raw greywater (Troiano, Beneduce, Gross, & Ronen, 2018). Some tetracycline-resistant isolates were also resistant to amoxicillin, ciprofloxacin, and kanamycin (Troiano et al., 2018). While to the author's knowledge no previous studies from the West Bank have investigated the prevalence of antibiotic-resistant bacteria in greywater treatment systems, antibiotic-resistant infections have been observed in the Palestinian population (Adwan, Jarrar, Abu-Hijleh, Adwan, & Awwad, 2014; Adwan et al., 2013; Al-Masri, Abu-Hasan, & Jouhari, 2016; B. Issa & Adwan, 2016). Thus, it would not be surprising to find resistant bacteria in household greywater in the West Bank.

Chemical Contaminants of Emerging Concern in Wastewater

Chemical contaminants can also survive the wastewater treatment process. Contaminants of emerging concern (CECs), such as pharmaceuticals, herbicides, and personal care products, have been noted in wastewater sources (Batt et al., 2007; Eriksson et al., 2003; Kulkarni et al., 2017). Antibiotics critical to human medicine, including ampicillin, azithromycin, ciprofloxacin, erythromycin, sulfamethoxazole, tetracycline, and vancomycin have been detected in effluent from WWTPs treating to the tertiary level in the US and Saudi Arabia (Alidina et al., 2014; Batt et al., 2007; Kulkarni et al., 2017; Panthi et al., In Press). Additionally, agricultural herbicides such as atrazine and alachlor have been identified in tertiary-treated municipal wastewater in the US and Saudi Arabia (Alidina et al., 2014; Panthi et al., In Press).

There is limited literature regarding contaminants of emerging concern in greywater. Studies of greywater in Europe identified the presence of the antibiotic triclosan as well as pesticides attributed to delousing shampoos (Donner et al., 2010; Eriksson et al., 2003; Eriksson & Donner, 2009; Zedek et al., 2015). One study evaluating greywater-irrigated soils in the United States showed that these soils contain higher levels of triclosan and triclocarban compared to freshwater-irrigated soils (Negahban-Azar, Sharvelle, Stromberger, Olson, & Roesner, 2012). It is likely that antibiotics in greywater have not been fully investigated as blackwater is considered the primary source of antibiotics in wastewater streams; however, this assumption neglects the potential for antibiotics to enter the greywater stream via personal care products, the washing of diapers, urinating while bathing, and improper disposal of antibiotics, among other routes. In addition, previous work has shown that common greywater treatment methods, such as constructed wetlands, inconsistently remove contaminants like antibiotics and herbicides (He et al., 2018; Huang et al., 2015; Matamoros, Puigagut, García, & Bayona, 2007).

Chemical Contaminants of Emerging Concern in Wastewater in Israel and the West Bank

There is minimal research regarding the presence of contaminants of emerging concern in wastewater in Israel or the West Bank. Only one known study has been conducted investigating antibiotics and herbicides in large-scale wastewater treatment plants in Israel and the West Bank, and that study was limited to triclosan and atrazine (Dotan et al., 2016). The study consistently found triclosan but did not identify atrazine in any samples (Dotan et al 2016). Research regarding antibiotic use and misuse among populations in the West Bank has noted practices such as taking antibiotics for viral infections, over-the-counter purchasing of antibiotics, and saving leftover antibiotics at home (Al Baz, Law, & Saadeh, 2018; Sweileh, 2004; Tayem et al., 2013; Sa'ed H. Zyoud et al., 2015). This high presence and utilization of antibiotics may drive the detection of antibiotic residues in household greywater.

Unanswered Questions About Off-grid Irrigation Water Quality and Acceptance of Reused Wastewater in Israel and the West Bank

As evidenced above, there is a dearth of knowledge regarding the quality of agricultural irrigation water from small-scale, off-grid greywater treatment systems in the West Bank, Palestinian Territories, specifically as it pertains to antibiotic-resistant bacteria and contaminants of emerging concern. Given the known health threats posed by bacterial pathogens and antibiotic-resistant bacteria, these data are needed to understand the potential health risks posed by irrigating with treated greywater. Additionally, there is a need for more research investigating overall wastewater reuse acceptance in both Israel and the Palestinian Territories. Understanding how and why the public differs regarding its acceptance of various forms of reuse is critical to determining if such projects will be accepted and what kinds of information the public needs.

To my knowledge, no previous studies have collected long-term data on the presence of antibiotic residues and antibiotic-resistant bacteria in greywater influent and treated effluent, and specifically no studies have investigated these contaminants in the West Bank, Palestinian Territories. Additionally, to my knowledge there are no studies comparing wastewater reuse acceptance between Palestinians and Israelis. The three manuscripts included in this dissertation seek to assess Israeli and Palestinian consumer acceptance of wastewater reuse, as well as evaluate greywater influent and treated effluent for antibiotic residues, overall bacteria levels, and presence of antibiotic-resistant bacteria.

Chapter 3: Antibiotic and herbicide concentrations in household greywater reuse systems and pond water used for food crop irrigation: West Bank, Palestinian Territories

Hillary A. Craddock[a], Suraj Panthi[a], Younes Rjoub[b], Clive Lipchin[b], Amir Sapkota[a], Amy R. Sapkota[a]

[a]Maryland Institute for Applied Environmental Health, University of Maryland School of Public Health, College Park, MD, USA

[b]Arava Institute for Environmental Studies, Ketura, Israel

Abstract

Introduction: Greywater is increasingly treated and reused in off-grid communities in the Middle East and other water scarce regions of the world. However, little is known about the water quality of these reuse systems. In particular, there is a dearth of data regarding levels of antibiotics and herbicides in off-grid greywater treatment systems.

Materials and Methods: Household greywater samples (influent and effluent, n=46) and irrigation pond water samples (n=12) were collected from four small farms in the West Bank, Palestinian Territories from October 2017 to June 2018. All samples were analyzed using high performance liquid chromatograph tandem mass spectrometry

(LC-MS/MS) for the following antibiotics and herbicides: alachlor, ampicillin, atrazine, azithromycin, ciprofloxacin, erythromycin, linezolid, oxacillin, oxolinic acid, penicillin G, pipemidic acid, sulfamethoxazole, triclocarban, tetracycline, triflualin, and vancomycin. Data were analyzed using descriptive statistics and repeated-measures ANOVA.

Results: All tested antibiotics and herbicides were detected in greywater influent samples at concentrations ranging from 1.3-1,592.9 ng/L and 3.1-22.4 ng/L, respectively. When comparing influent to effluent concentrations, removal was observed for azithromycin, alachlor, linezolid, oxacillin, penicillin G, pipemidic acid, sulfamethoxazole, triclocarban, and vancomycin. Removal was not observed for atrazine, ciprofloxacin, erythromycin, oxolinic acid, tetracycline, and trifluralin. Pond water also contained the majority of tested contaminants, but at generally lower concentrations than effluent.

Discussion and Conclusion: To our knowledge, this is the first description of an extensive array of antibiotics and herbicides detected in household greywater from off-grid treatment systems. The consistent detection of antibiotics in greywater is concerning since these contaminants could potentially contribute to the selection of antibiotic-resistant bacteria in soil and irrigated products in contact with reused greywater. Improved designs of off-grid greywater reuse systems that can more effectively remove antibiotics and other chemical contaminants is warranted.

Introduction

The Middle East continues to face water scarcity challenges in key food production areas (Dare et al., 2017; Shomar & Dare, 2015). To adapt, wastewater is commonly used for food crop irrigation (Faour-Klingbeil & Todd, 2018; Shomar & Dare, 2015). Due to the lack of capacity for large-scale wastewater reuse, small-scale, off-grid greywater treatment solutions have been implemented in the West Bank, Palestinian Territories (McIlwaine & Redwood, 2011). Greywater is household water from all sources other than toilets, and includes water from kitchens, bathing, clothes washing, and hand washing (McIlwaine & Redwood, 2011). As greywater from households does not contain sewage or industrial effluent, it is considered to have lower levels of bacteria, pharmaceuticals, and other pollutants compared to municipal wastewater (Benami, Gillor, & Gross, 2016; Gross, Kaplan, & Baker, 2007; Maimon, Friedler, & Gross, 2014; Ronen, Guerrero, & Gross, 2010). However, few studies have evaluated the quality of greywater as it pertains to contaminants of emerging concern (Donner et al., 2010; Eriksson et al., 2003; Zedek et al., 2015).

Two common methods for treating greywater in low-resource settings are constructed wetlands and upflow gravel filters. Constructed wetlands are typically a series of containers filled with gravel and plants. Breakdown of contaminants is achieved through exposure to biofilms and ultraviolet light and via uptake by plants (phytoremediation) (Little, 2014). Upflow gravel filtration systems combine passive gravel filtration with active pumping of wastewater through anaerobic tanks (Harman et al., 2017). These off-grid systems, while cheaper and easier to implement in low-resource settings, often have challenges related to factors including bad odors and unacceptably high levels of bacteria (Alfiya, Gross, Sklarz, & Friedler, 2013; Harman et al., 2017; Little, 2014). However, data regarding the efficiency of these systems in removing contaminants of emerging concern is limited (Breitholtz et al., 2012; Chen, Wei, et al., 2016; He et al., 2018; Huang et al., 2015).

While several studies, including those conducted in Israel and the West Bank, have investigated antibiotic concentrations in the influent and effluent of large-scale wastewater treatment plants (Dotan et al., 2016; Kulkarni et al., 2017, Panthi et al., In Press), the investigation of greywater for contaminants of emerging concern is thus far minimal. Studies of greywater in Europe identified the presence of the antibiotic triclosan and pesticides attributed to delousing shampoos (Donner et al., 2010; Eriksson et al., 2003; Eriksson & Donner, 2009; Zedek et al., 2015). Similarly, one study evaluating greywater-irrigated soils in the United States showed that these soils contain higher levels of triclosan and triclocarban than freshwater-irrigated soils (Negahban-Azar, Sharvelle, Stromberger, Olson, & Roesner, 2012).

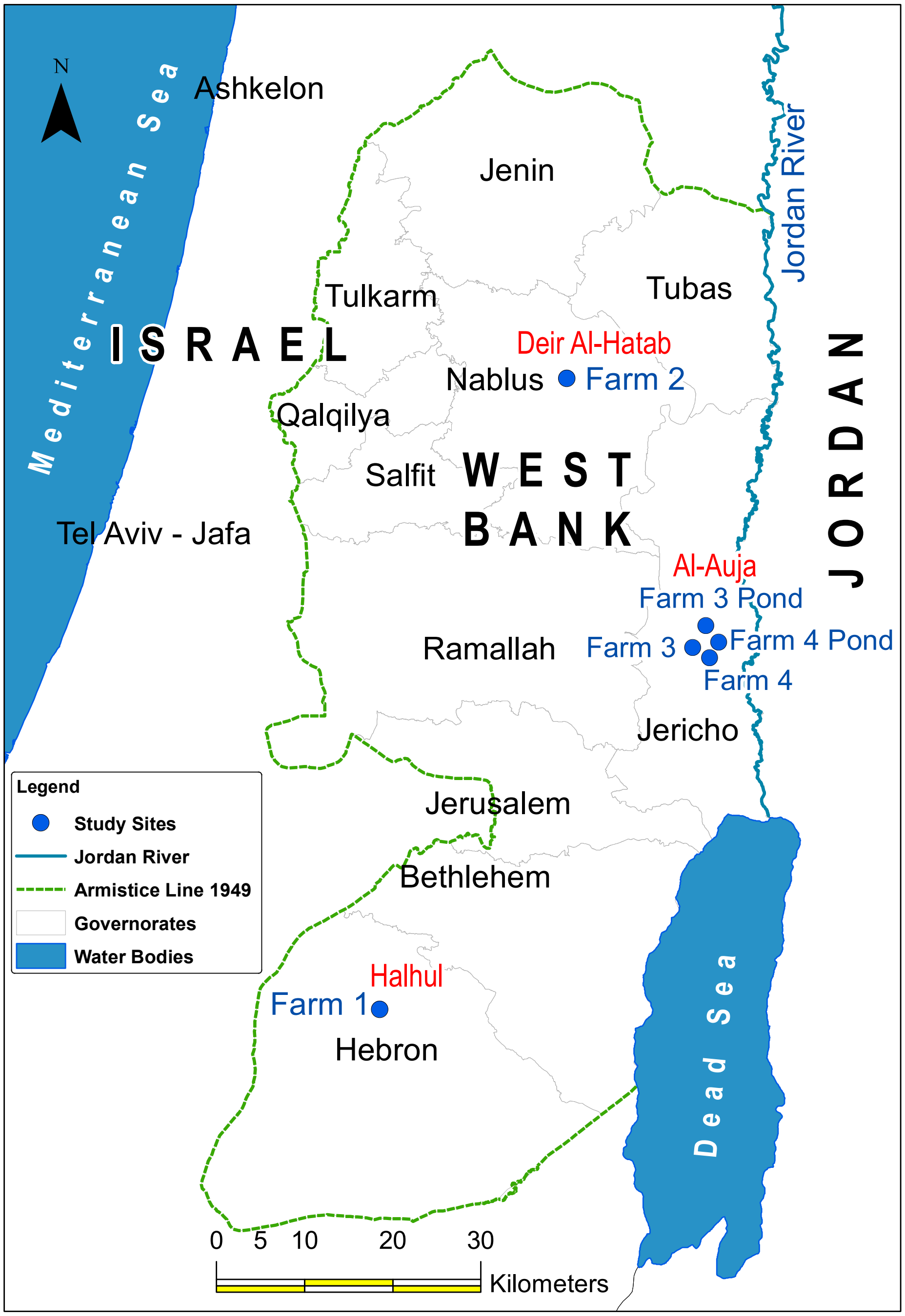
To address the dearth of literature regarding levels of antibiotics and herbicides in greywater and the effectiveness of greywater treatment systems, the goal of this study was to quantify the concentrations of these contaminants in the influent and effluent of small-scale, off-grid greywater treatment systems in the West Bank, Palestinian Territories over a period of nine months. Understanding the presence of these contaminants in greywater, as well as their removal via small-scale, off-grid systems, is relevant to farmers in the West Bank as well as farmers residing in other water scarce regions who may be implementing similar greywater reuse systems for food crop irrigation.

Materials and Methods

Study Sites

Four farms in the West Bank, Palestinian Territories, were included in the study (Figure 1). Farm 1, located in the Hebron Governorate, utilizes a constructed wetlands greywater reuse system. Farm 2, located in the Nablus Governorate, also utilizes a constructed wetlands greywater reuse system. Farms 3 and 4 are located in the Jericho Governorate and both utilize upflow gravel filtration greywater reuse systems as well as holding ponds for pumped groundwater. A variety of crops are irrigated with the greywater, including but not limited to: date trees, citrus trees, cucumbers, tomatoes, and zucchini. Drip irrigation is used by all farmers on all crops. Farmers were included in this study based on their prior association with projects launched by members of our team at the Arava Institute for Environmental Studies (Ketura, Israel). Farmers were contacted by phone by a native Arabic speaker to ask if they would be willing to have multiple water samples collected over time from their systems.

Figure 1: Map of study sites, West Bank, Palestinian Territories. Farms 1 and 2 utilize constructed wetlands greywater reuse systems. Farms 3 and 4 utilize upflow gravel filtration greywater reuse systems and holding ponds for pumped groundwater.



Sample Collection

Water samples were collected during eight sampling events that occurred over nine months (October 2017 - June 2018). Timing of sampling was dependent on the availability of farmers as well as political unrest. Additionally, the Farm 2 system clogged and needed to be taken offline for repair in November and thus, only one influent-effluent pair was collected from this system. All samples were collected in sterile 250mL Nalgene ® Wide Mouth Bottles (VWR International, Radnor PA) that had been pre-treated with hydrochloric acid solution (2:1 dilution of HPLC-grade water to HPLC-grade hydrochloric acid). Samples were transported to the laboratory at 4° C and stored at -20° C until processing. A total of 58 samples were included in this analysis: 23 influent samples, 23 effluent samples, and 12 pond water samples.

Extraction and Analysis of Antibiotic and Herbicide Concentrations

All samples were analyzed for the following twelve antibiotics, three herbicides, and one stimulant, using a previously published method with modifications (Sapkota, Heidler, & Halden, 2007, Panthi et al., In Press): alachlor (ALA), ampicillin (AMP), atrazine (ATR), azithromycin (AZI), caffeine (CAF), ciprofloxacin (CIP), erythromycin (ERY), linezolid (LIN), oxacillin (OXA), oxolinic acid (OXO), penicillin G (PEN), sulfamethoxazole (SUL), triclocarban (TCC), tetracycline (TET), trifluralin (TRI), and vancomycin (VAN).

A 10 µL aliquot of 10 µg/mL HPLC-grade methanol stock solution containing each of the following internal standards was added to a 200 mL aliquot of each sample, followed by thorough mixing and equilibration, for a final concentration of

100 ng/mL: Alachlor -d13, Atrazine-d5, Benzyl Penicillinate-d7 Potassium Salt (Penicillin G-d7 Potassium Salt), Caffeine-13C3, Linezolid-d3, Oxolinic Acid-d5, Tetracycline-d6, and Trichlorocarbanilide-13C6 (Triclocarban-13C6).

All samples were then extracted using Oasis HLB (60 cc) cartridges (Milford, MA, USA) on a Visiprep 12-port vacuum manifold (Sigma Aldrich, St. Louis MO, USA) with minimal vacuum. Cartridges were conditioned with 5 mL of methanol, followed by a 3mL rinse with HPLC grade water. Samples were loaded onto the HLB columns at a flowrate of approximately 1 mL/min. After sample loading, the cartridges were frozen at -20°C, then shipped from Israel to the US on dry ice. Upon arrival in the US, the cartridges were thawed at room temperature then eluted with 5 mL of a 50:50 methanol/acetone mix followed by 3 mL of methanol with 0.1% formic acid. The extracts were dried under gentle nitrogen flow at 40°C. Finally, the samples were reconstituted with 1 mL of 90:10 water:methanol mix and transferred to 1.5 mL autosampler vials for LC-MS/MS analysis.

An autoinjector was used to inject 5 µL of sample onto an Agilent 1290 Infinity II HPLC system coupled with an Agilent 6470 QQQ tandem mass spectrometer. Chromatographic separation was achieved using an Agilent C18 Zorbax Eclipse Plus 3.0 x 50 mm, 1.8 micron column. A gradient mobile phase (flowrate 0.8 mL/min) consisted of 99% A (95:5 water:acetonitrile with 0.1% formic acid) at 0 min and switched to 90% B (95:5 acetonitrile:water with 0.1% formic acid) at 7.5 min with a total run time of 12 min. Analytes were introduced into the mass spectrometer using an electrospray ionization probe that operated on dynamic MRM mode, which allowed polarity switching between negative and positive ionization mode. A 7-point calibration curve ranging from 0 to 200 ng/mL was used to quantify the analytes. After every 10 sample injections, a blank and spiked standard were injected for QA/QC purposes. For limits of detection, parameters, and rate of recovery on individual analytes, see Supplemental Table 1.

Statistical analysis

Descriptive statistics were calculated to compare the detection frequencies and concentrations of the tested antibiotics and herbicides between influent and effluent and between effluent and pond water. To test whether the mean concentrations of antibiotics were significantly different across sample types, concentration values were log-transformed to account for non-normality and a repeated measures ANOVA was utilized. All concentration values that were below the limit of detection (LOD) were replaced with ½ LOD. Removal was assessed by calculating the ratio of effluent concentration to influent concentration. P-values <0.05 were considered statistically significant. Statistical analyses were carried out in SAS 9.4 (Cary, NC, USA). Due to sample size, analyses were conducted on all four farms together. Plots were created in R (Version 3.5.1, The R Foundation for Statistical Computing, Vienna, Austria), and the map was designed on ArcGIS ArcMap (Version 10.4.1, Esri, Redlands CA, USA).

Results

Presence and Concentrations of Antibiotics and Herbicides in Influent and Effluent

Overall, tested antibiotics and herbicides were detected in influent samples at observed concentrations ranging from 1.3-1,592.9 ng/L and 3.1-22.4 ng/L, respectively (Table 1). Caffeine was detected in influent samples at concentrations ranging from 2,855.7-4.1x10 ^{5} ng/L (Table 1). Tested antibiotics and herbicides were detected in effluent samples at concentrations ranging from 1.3- 2,042.8 ng/L and 3.3-329.2 ng/L, respectively (Table 1). Caffeine was detected in effluent samples at concentrations ranging from 40.4-1.9x10 ^{5} ng/L (Table 1).

As seen in Table 1, the highest detection frequencies across all influent samples recovered from all four farms were for caffeine (100%), atrazine (82.6%), pipemidic acid (60.9%), azithromycin (56.5%), and oxolinic acid (56.5%). When looking at the detection frequencies of these analytes in effluent compared to influent, atrazine and oxolinic acid were detected more frequently in effluent (95.7% and 73.9%, respectively) and pipemidic acid and azithromcyin were detected less frequently in effluent (39.1% and 4.4% respectively). The lowest detection frequencies across all influent samples recovered from all four farms were for erythromycin (13%), tetracycline (17.4%), and trifluralin (17.4%). In comparing the detection frequencies of these analytes in effluent vs influent, erythromycin and trifluralin were detected more frequently in effluent than influent (17.4% and 21.7% respectively), whereas tetracycline was detected less frequently in effluent (8.7%)

than influent. Vancomycin was detected in 26.1% of influent samples but 0% of effluent samples.

Observed removal of antibiotics and herbicides during greywater treatment

The ratio of mean effluent/influent concentrations, which indicates how well an analyte is removed, varied across analytes. Ratios < 1.0 indicated reductions in concentrations from influent to effluent and these ratios were observed for azithromycin (0.1), vancomycin (0.1), triclocarban (0.1), caffeine (0.3), sulfamethoxazole (0.4), pipemidic acid (0.4), oxacillin (0.5), penicillin G (0.6), linezolid (0.6), and alachlor (0.6). Ratios > 1.0, that indicate increases in concentrations from influent to effluent, were observed for tetracycline (1.2), ciprofloxacin (1.2), trifluralin (1.2), oxolinic acid (1.4), erythromycin (1.6), and atrazine (2.5). These differences are visualized in Figures 2, 3, and 4.

When comparing mean influent to effluent concentrations, four compounds were found to be statistically significantly higher in influent compared to effluent: caffeine (p-value=0.02), pipemidic acid (p-value=0.03), vancomycin (p-value=0.01), and azithromycin (p-value<0.0001). Oxolinic acid concentrations were slightly higher in influent compared to effluent; however, the difference was marginally significant (p-value=0.056).

Comparing Treated Effluent to Pond Water

The pond water, which is pumped groundwater that is stored at the surface, was generally characterized by lower concentrations of antibiotics compared to both influent and effluent samples (Figure 2). This was demonstrated by ratios of mean pond water/effluent concentrations <1 for the following compounds: atrazine (0.5), ciprofloxacin (0.5), erythromycin (0.1), linezolid (0.8), oxacillin (0.02), oxolinic acid (0.5), penicillin G (0.2), pipemidic acid (0.4), sulfamethoxazole (0.1), tetracycline (0.005), and caffeine (0.005). Exceptions included azithromycin (3.3), triclocarban (1.7), trifluralin (2.0), and alachlor (1.1), all of whom had a higher mean concentration in pond water than in effluent. Vancomycin was detected in neither pond water nor effluent.

Regarding detection frequencies, erythromycin, oxacillin, sulfamethoxazole, tetracycline, and vancomycin were not detected in any pond water samples. In addition, the following compounds were characterized by relatively low detection frequencies across all pond water samples: penicillin G (8.3%), azithromycin (16.7%), pipemidic acid (16.7%), and alachlor (25.0%). The highest detection frequencies in pond water samples were for caffeine (100%), atrazine (100%), oxolinic acid (83.3%), and triclocarban (66.7%). These differences are visualized in Figures 2, 3, and 4. When comparing effluent to pond samples, three compounds were found to be statistically significantly lower in pond samples: caffeine (*p*-value=0.0002), penicillin G (*p*-value=0.05), and sulfamethoxazole (*p*-value=0.03).

Table 1: Detection frequencies and concentrations of herbicides (alachlor (ALA), atrazine (ATR), and trifluralin (TRI)), antibiotics (azithromycin (AZI), ciprofloxacin (CIP), erythromycin (ERY), linezolid (LIN), oxacillin (OXA), oxolinic acid (OXO), penicillin G (PEN), sulfamethoxazole (SUL), triclocarban (TCC), tetracycline (TET), and vancomycin (VAN)), and caffeine (CAF) in greywater influent (n=23), constructed wetland- and upflow gravel filtration system-treated effluent samples (n=23), and irrigation pond water samples (n=12), including the percentage of samples testing above the limit of detection, mean sample concentrations (in ng/L), and range of observed sample concentrations.

Farm 1		ALA	ATR	TRI	AZI	CIP	ERY	LIN	OXA	OXO	PEN	PIP	SUL	TCC	TET	VAN	CAF
Influent	**% > LOD**	**14.3**	**85.7**	**12.5**	**28.6**	**42.9**	**0**	**42.9**	**28.6**	**71.4**	**28.6**	**57.1**	**42.9**	**28.6**	**14.3**	**14.3**	**100**
n = 7	Mean (SD)	3.5 (8.4)	10.2 (4.6)	0.4 (1.1)	7.6 (12.4)	30.8 (37.8)	.	2.6 (4.7)	77.9 (153.7)	44.4 (52.7)	16.6 (27.8)	71.7 (113.4)	8.3 (13.1)	387.2 (674.3)	47.8 (124.9)	1.6 (3.4)	7.1×10^4 (5.3×10^4)
	Range	0.3 - 22.4	0.1 - 14.0	0.01 - 3.2	0.3 - 26.0	0.4 - 71.7	.	0.3 - 13.1	0.9 - 408.3	0.5 - 150.7	0.7 - 66.4	0.4 - 319.9	0.5 - 36.3	0.7 - 1592.9	0.5 - 331.07	0.3 - 9.2	1.3×10^4 - 1.7×10^5
Effluent	**% > LOD**	**0**	**100**	**0**	**0**	**71.4**	**14.3**	**28.6**	**85.7**	**71.4**	**28.6**	**57.1**	**28.6**	**28.6**	**14.3**	**0**	**100**
n = 7	Mean (SD)	.	11.6 (0.7)	.	.	54.8 (37.5)	1.5 (3.5)	0.6 (0.5)	134.4 (84.2)	40.9 (40.2)	15.9 (26.5)	35.0 (32.4)	3.1 (4.4)	42.2 (71.0)	292.3 (771.9)	.	6996.4 (8609.0)
	Range	.	10.8-12.8	.	.	0.4 - 87.4	0.2 - 9.4	0.3 - 1.4	0.9 - 265.5	0.5 - 118.4	0.7 - 63.1	0.4 - 62.5	0.5 - 10.4	0.7 - 154.0	0.5 - 2042.7	.	91.4 - 2.0×10^4
*Farm 2**																	
Influent	**% > LOD**	**0**	**100**	**100**	**0**	**0**	**0**	**0**	**0**	**100**	**0**	**100**	**100**	**100**	**0**	**0**	**100**
n = 1	Mean (SD)	.	10.6 (.)	3.2 (.)	.	.	.	.	.	151.9 (.)	.	71.7 (.)	48.0 (.)	65.8 (.)	.	.	9.6×10^4 (.)
Effluent	**% > LOD**	**0**	**100**	**0**	**0**	**100**	**0**	**0**	**100**	**0**	**100**	**0**	**100**	**0**	**0**	**0**	**100**
n = 1	Mean (SD)	.	10.7 (.)	.	.	71.0 (.)	.	.	103.7 (.)	.	46.0 (.)	.	18.1 (.)	.	.	.	1.6×10^4 (.)
Farm 3																	
Influent	**% > LOD**	**42.9**	**100**	**14.3**	**57.1**	**28.6**	**42.9**	**42.9**	**28.6**	**42.9**	**85.7**	**71.4**	**14.3**	**42.9**	**28.6**	**42.9**	**100**
n = 8	Mean (SD)	5.7 (6.7)	11.3 (0.8)	0.7 (1.9)	16.1 (14.8)	22.3 (37.4)	4.5 (5.5)	0.8 (0.6)	37.3 (63.2)	22.1 (28.9)	56.7 (31.6)	69.9 (77.4)	3.6 (8.1)	51.4 (66.1)	71.6 (124.5)	4.0 (4.6)	1.7×10^5 (9.7×10^4)
	Range	0.3 - 13.6	10.7-12.5	0.01 - 5	0.3 - 31.4	0.4 - 78.7	0.2 - 12.8	0.3 - 1.7	0.9 - 148.2	0.5 - 72.0	0.7 - 95.0	0.4 - 231.6	0.5 - 22	0.7 - 145.6	0.5 - 297.6	0.3 - 9.4	5.1×10^4 - 3.0×10^5
Effluent	**% > LOD**	**37.5**	**87.5**	**25**	**0**	**62.5**	**37.5**	**75**	**0**	**62.5**	**12.5**	**50**	**37.5**	**12.5**	**12.5**	**0**	**100**
n = 7	Mean (SD)	4.8 (6.2)	49.3 (113.1)	0.9 (1.7)	.	44.8 (36.8)	5.2 (7.7)	1.1 (0.5)	.	72.1 (100.8)	6.2 (15.7)	30.2 (31.9)	4.2 (6.0)	8.9 (23.0)	9.6 (25.6)	.	9.7×10^4 (2.0×10^4)
	Range	0.3 - 12.9	0.1 - 329.2	0.01 - 3.9	.	0.4 - 73.8	0.2 - 20.7	0.3 - 1.6	.	0.5 - 296.0	0.7 - 45.1	0.4 - 60.4	0.5 - 17.0	0.7 - 65.8	0.5 - 72.9	.	4.7×10^4 - 1.9×10^5
Pond	**% > LOD**	**0**	**100**	**66.7**	**16.7**	**50**	**0**	**50**	**0**	**83.3**	**0**	**0**	**0**	**66.7**	**0**	**0**	**100**
n = 6	Mean (SD)	.	10.7 (0.10)	2.8 (2.6)	4.9 (11.1)	35.4 (38.3)	.	0.8 (0.6)	.	27.0 (17.8)	.	.	.	47.7 (36.5)	.	.	136.1 (174.1)
	Range	.	10.6-10.9	0.01 - 6.9	0.3 - 27.6	0.4 - 70.5	.	0.3 - 1.5	.	0.5 - 56.2	.	.	.	0.7 - 76.0	.	.	23.8 - 464.6
Farm 4																	
Influent	**% > LOD**	**37.5**	**62.5**	**12.5**	**87.5**	**75**	**0**	**62.5**	**75**	**50**	**50**	**50**	**75**	**37.5**	**12.5**	**25**	**100**
n = 7	Mean (SD)	6.2 (8.5)	8.0 (6.9)	0.9 (2.5)	38.8 (21.8)	72.2 (61.3)	.	1.9 (1.6)	174.8 (116.7)	25.7 (29.9)	43.0 (58.2)	43.8 (55.8)	18.5 (25.6)	330.9 (482.5)	111.4 (313.6)	3.0 (5.1)	1.1×10^4 (1.3×10^5)
	Range	0.3 - 20.7	0.1 - 18.4	0.01 - 7	0.3 - 77.7	0.4 - 197.8	.	0.3 - 3.8	0.9 - 293.9	0.5 - 78.6	0.7 - 162.8	0.4 - 158.1	0.5 - 79.2	0.7 - 1189.9	0.5 - 887.5	0.3 - 12.7	2855.7 - 4.1×10^5
Effluent	**% > LOD**	**28.6**	**100**	**42.9**	**14.3**	**71.4**	**0**	**85.7**	**14.3**	**100**	**85.7**	**14.3**	**85.7**	**42.9**	**0**	**0**	**100**
n = 8	Mean (SD)	3.7 (5.7)	11.5 (0.6)	2.3 (3.1)	3.9 (9.4)	52.1 (35.3)	.	1.5 (0.5)	17.3 (43.4)	45.0 (25.2)	40.3 (17.9)	8.8 (22.4)	7.5 (3.3)	37.4 (45.8)	.	.	1466.5 (1584.6)
	Range	0.3 - 12.3	10.7 - 12.4	0.01 - 7.8	0.3 - 25.1	0.4 - 75.9	.	0.3 - 2.1	0.9 - 115.8	27.4 - 97.7	0.7 - 54.7	0.4 - 59.7	0.5 - 10.6	0.7 - 91.5	.	.	40.4 - 4892.5
Pond	**% > LOD**	**50**	**100**	**33.3**	**16.7**	**16.7**	**0**	**33.3**	**0**	**83.3**	**16.7**	**33.3**	**0**	**66.7**	**0**	**0**	**100**
n = 6	Mean (SD)	6.3 (6.5)	13.5 (3.9)	1.1 (1.7)	4.5 (10.3)	12.1 (28.5)	.	0.7 (0.5)	.	21.0 (10.9)	7.6 (16.9)	20.0 (30.4)	.	46.6 (35.6)	.	.	228.0 (357.7)
	Range	0.3 - 12.6	10.8 - 19.6	0.01 - 3.7	0.3 - 25.5	0.4 - 70.4	.	0.3 - 1.4	.	0.5 - 33.3	0.7 - 42	0.4 - 59.3	.	0.7 - 72.8	.	.	25.7 - 953.4
All Farms		**ALA**	**ATR**	**TRI**	**AZI**	**CIP**	**ERY**	**LIN**	**OXA**	**OXO**	**PEN**	**PIP**	**SUL**	**TCC**	**TET**	**VAN**	**CAF**
Influent	**% > LOD**	**30.4**	**82.6**	**17.4**	**56.5**	**47.8**	**13**	**47.8**	**43.5**	**56.5**	**52.2**	**60.9**	**47.8**	**39.1**	**17.4**	**26.1**	**100**
n = 23	Mean (SD)	5.0 (7.5)	9.8 (4.8)	0.8 (1.9)	20.7 (21.3)	41.3 (50.4)	1.5 (3.5)	1.7 (2.7)	95.9 (125.5)	35.8 (44.7)	37.3 (43.5)	61.4 (79.4)	12.1 (19.4)	251.4 (470.9)	75.1 (201.9)	2.8 (4.3)	1.2×10^5 (1.0×10^5)
	Range	0.3 - 22.4	0.1 - 18.4	0.01 - 7.0	0.3 - 77.7	0.4 - 197.8	0.2 - 12.8	0.3 - 13.1	0.9 - 408.3	0.5 - 151.9	0.7 - 162.8	0.4 - 319.9	0.5 - 79.2	0.7 - 1592.9	0.5 - 887.5	0.3 - 12.7	2855.7 - 4.1×10^5
Effluent	**% > LOD**	**21.7**	**95.7**	**21.7**	**4.4**	**69.6**	**17.4**	**60.9**	**34.8**	**73.9**	**43.5**	**39.1**	**52.2**	**26.1**	**8.7**	**0**	**100**
n = 23	Mean (SD)	2.9 (5.0)	24.6 (66.4)	1.0 (2.1)	1.4 (5.2)	51.2 (34.5)	2.4 (5.2)	1.0 (0.6)	51.0 (77.8)	51.3 (64.5)	21.3 (30.0)	23.9 (30.0)	5.5 (5.6)	27.3 (48.7)	92.5 (425.4)	.	3.7×10^4 (5.3×10^4)
	Range	0.3 - 12.9	0.1 - 329.2	0.01 - 7.8	0.3 - 25.1	0.4 - 87.4	0.2 - 20.7	0.3 - 2.1	0.9 - 265.5	0.5 - 296	0.7 - 63.1	0.4 - 62.5	0.5 - 18.1	0.7 - 154.1	0.5 - 2042.8	.	40.4 - 1.9×10^5
Pond	**% > LOD**	**25**	**100**	**50**	**16.7**	**33.3**	**0**	**41.7**	**0**	**83.3**	**8.3**	**16.7**	**0**	**66.7**	**0**	**0**	**100**
n = 12	Mean (SD)	3.3 (5.4)	12.1 (3.0)	2.0 (2.3)	4.7 (10.2)	23.8 (34.4)	.	0.8 (0.5)	.	24.0 (14.4)	4.1 (11.9)	10.2 (22.9)	.	47.2 (34.4)	.	.	182.1 (272.5)
	Range	0.3 - 12.6	10.6 - 19.6	0.01 - 6.9	0.3 - 27.6	0.4 - 70.5	.	0.3 - 1.5	.	0.5 - 56.2	0.7 - 42	0.4 - 59.3	.	0.7 - 76	.	.	23.8 - 953.4

*Only one timepoint was collected for Farm 2, thus there is only one sample each for influent and effluent.

Figure 2: Concentrations (ng/L) of the following antibiotics in greywater influent (n=23), treated greywater effluent (n=23), and irrigation pond water (n=12) among four farms and two irrigation ponds in the West Bank, Palestinian Territories: azithromycin (AZI), ciprofloxacin (CIP), erythromycin (ERY), linezolid (LIN), oxacillin (OXA), oxolinic Acid (OXO), penicillin G (PEN), sulfamethoxazole (SUL), triclocarban (TCC), tetracycline (TET), and vancomycin (VAN).

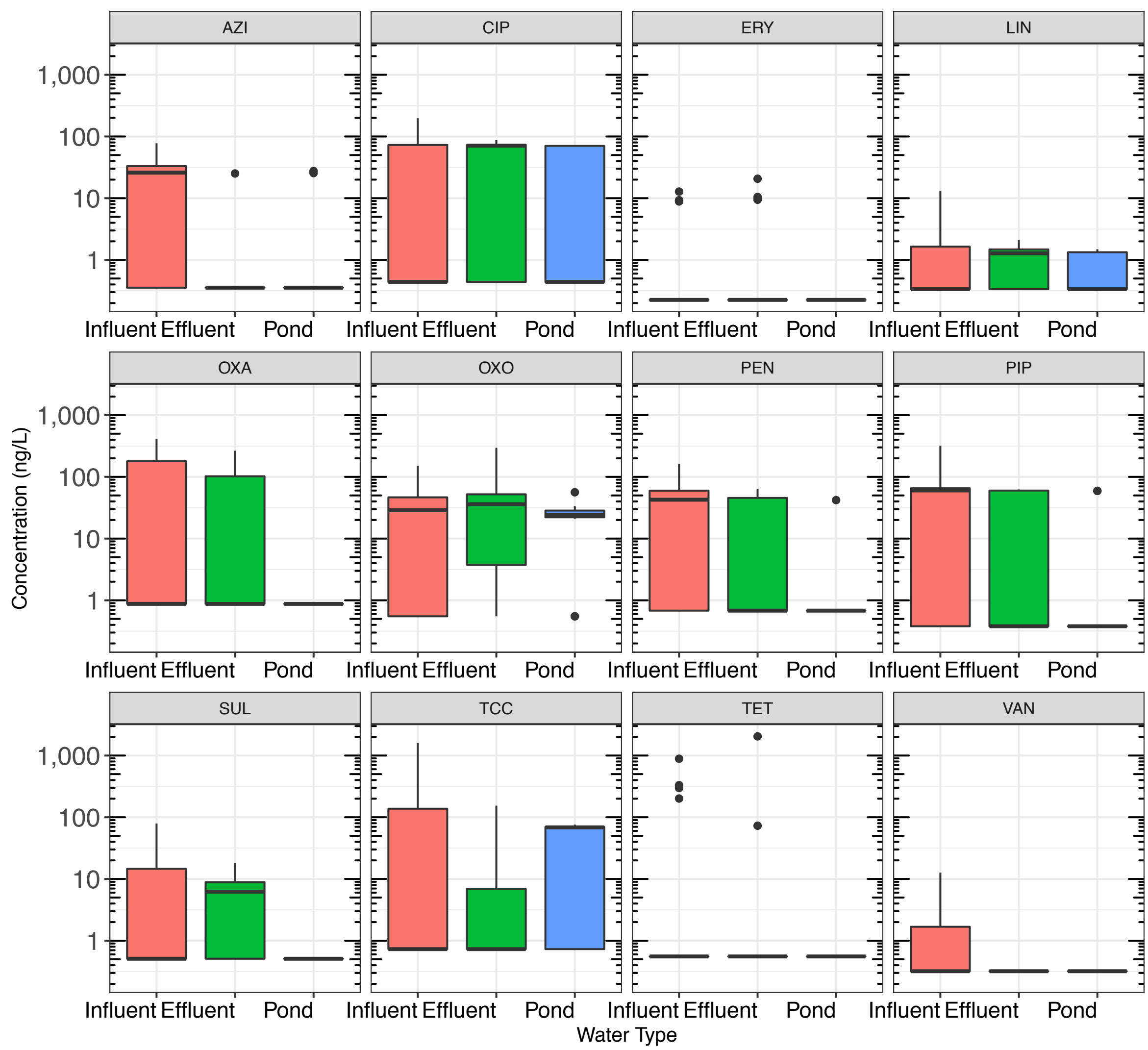


Figure 3: Concentrations (ng/L) of the following herbicides in greywater influent (n=23), treated greywater effluent (n=23), and irrigation pond water (n=12) among four farms and two irrigation ponds in the West Bank, Palestinian Territories: alachlor (ALA), atrazine (ATR), and trifluralin (TRI).

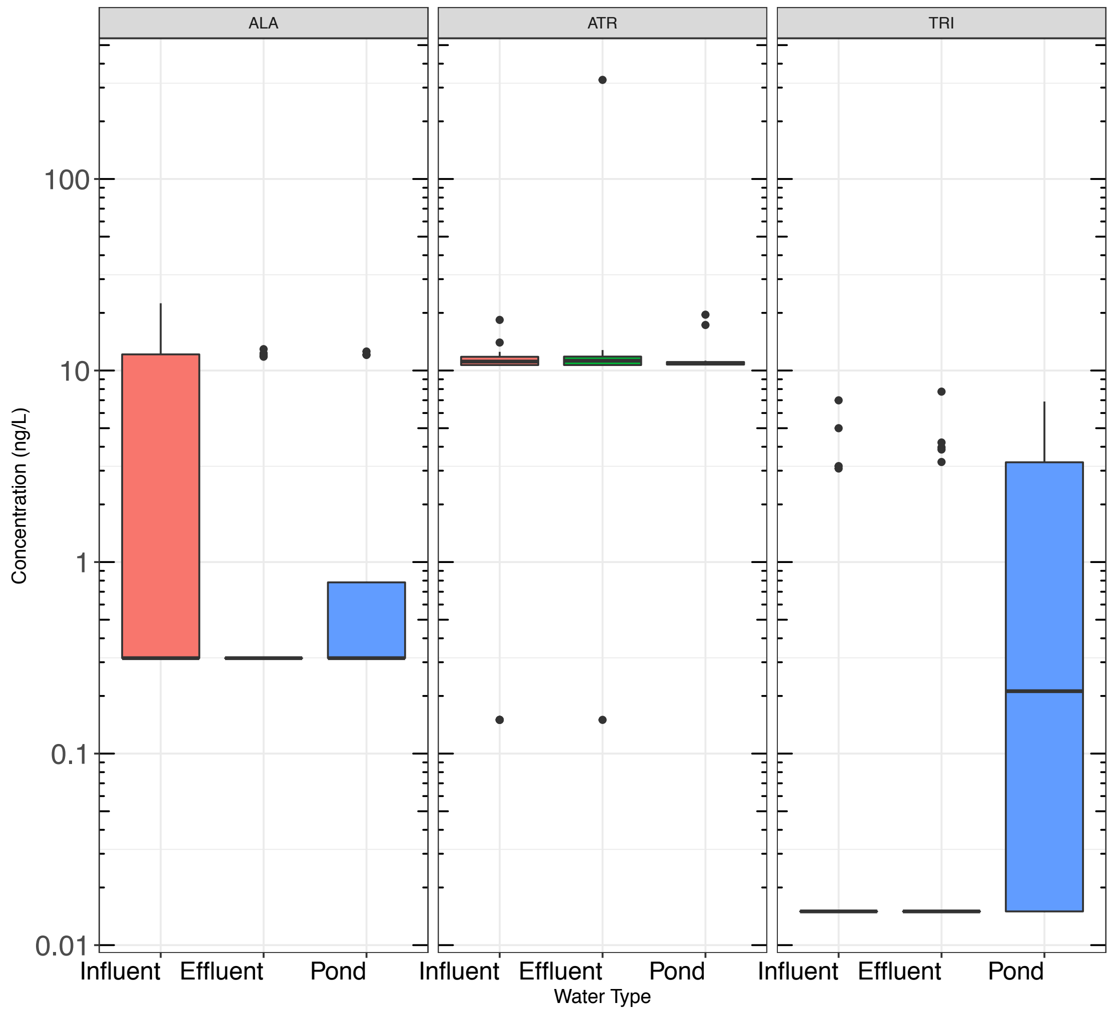
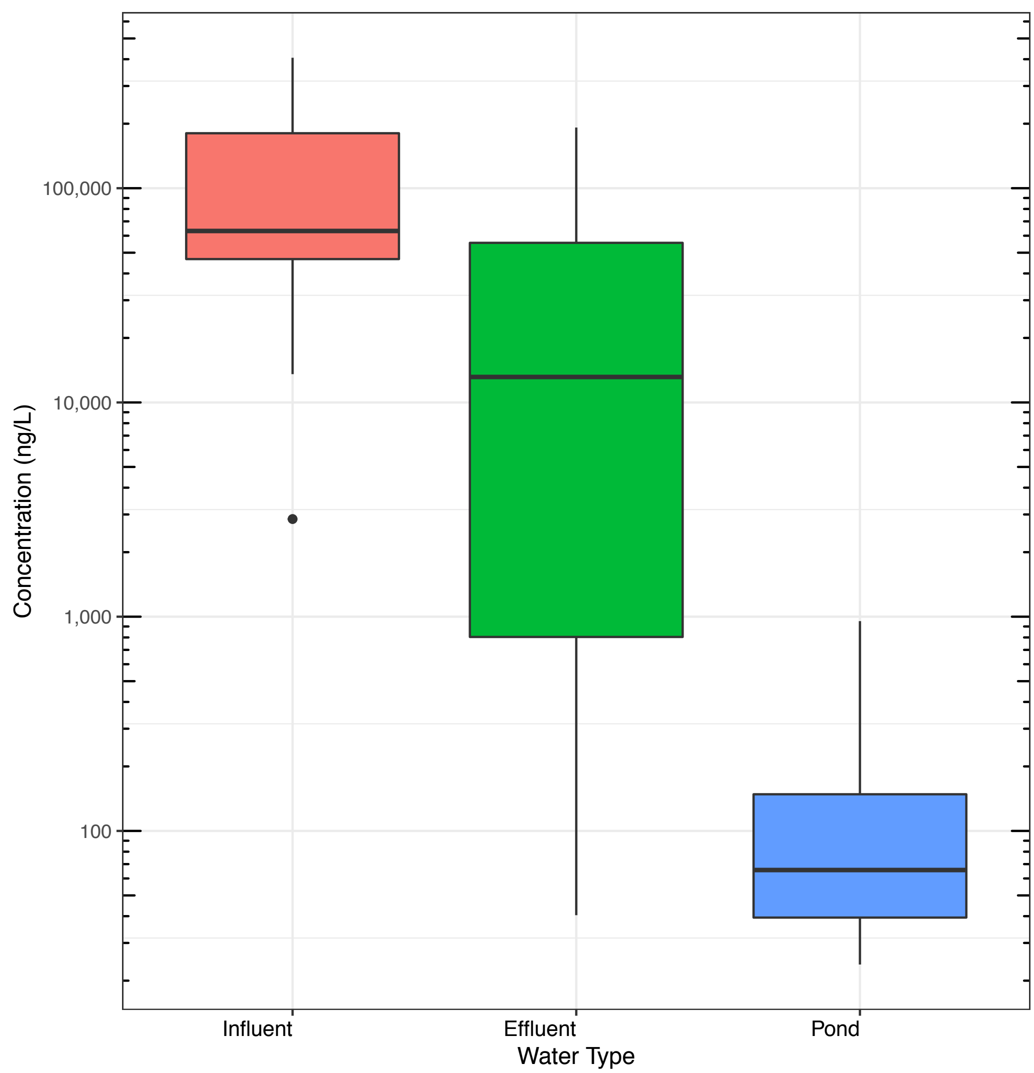


Figure 4: Concentrations of caffeine (ng/L) in greywater influent (n=23), treated greywater effluent (n=23), and irrigation pond water (n=12) among four farms and two irrigation ponds in the West Bank, Palestinian Territories.



Discussion

Summary of Study Findings

This study characterized four off-grid greywater treatment systems in the West Bank of the Palestinian Territories in order to assess contaminants of emerging concern, namely antibiotics and herbicides. Additionally, we studied the presence of these compounds in agricultural irrigation water holding ponds, thus providing context as to how treated greywater compares to the water the farmers would ordinarily use. Overall, we identified multiple antibiotics and herbicides in greywater influent, effluent treated via constructed wetlands and upflow gravel filtration systems, and irrigation pond water. The removal of these compounds during the treatment process was very mixed. Fewer antibiotics were detected in pond water than in greywater effluent.

Presence of Antibiotics in Influent

Prior studies in the West Bank identified high levels of consumer access to a wide variety of antibiotics, including via over-the-counter purchases and sharing of leftover antibiotics among family members (Abu Taha et al., 2016; Al Baz, Law, & Saadeh, 2018; Hajjaj, 2005; Sawalha, 2010; Sawalha, 2008; Tayem et al., 2013). Given the high levels of antibiotic use reported in the literature, it was expected that we would identify high antibiotic concentrations in household greywater.

The only antibiotic that has been investigated in greywater in the published literature is triclosan, as it is present in a wide range of consumer goods (Halden & Paull, 2005). However, we investigated triclocarban, an antibiotic with similar properties and uses as triclosan that has been found to co-occur with triclosan in surface waters (Halden & Paull, 2005). Our mean detected value of 251.4 ng/L triclocarban in influent samples is lower than previously published detection levels of triclosan in European greywater, which range from 500-5,900 ng/L (Eriksson et al., 2003; Zedek et al., 2015). Regarding other antibiotics, our detected concentrations of erythromycin and sulfamethoxazole are lower than those reported from municipal wastewater treatment plants (Chen et al., 2016; He et al., 2018).

Presence of Herbicides in Influent

The agricultural practices of farmers in the Palestinian Territories additionally suggest that herbicides may find their way into greywater streams (ARIJ - Applied Research Institute Jerusalem, 1995; Issa, Sham'a, Nijem, Bjertness, & Kristensen, 2010). Studies of farmer behavior in the West Bank and Gaza have noted behaviors such as storing pesticides in the home, preparing pesticides in the kitchen, washing and reusing pesticide containers at home, and bathing in the home after pesticide application (Yassin, Abu Mourad, & Safi, 2002; Zyoud et al., 2010). Donner et al. (2010) identified alachlor, atrazine, and trifluralin as potential contaminants in household greywater even in non-agricultural European households. Thus, our findings of these substances in agricultural households is expected (Donner et al., 2010).

Nevertheless, our detected levels of atrazine are lower than those observed in municipal wastewater in Sweden (Gros et al., 2017). Observing trifluralin was moderately surprising, as it was phased out in Israel in 2015 (Berman et al., 2017).

However, trifluralin has been noted to be persistent in the environment (European Union, 2012; Mamy, Barriuso, & Gabrielle, 2005), and to the authors' knowledge, it is not banned in Jordan, and therefore, the presence of trifluralin is likely due to some combination of farmers "stocking up" with trifluralin before the phase out in Israel, additional stocks coming in from Jordan, and environmental persistence.

Implications for International Development and Future Research

As noted above, we identified detectable levels of antibiotics in treated greywater effluent, as well as variable removal efficiencies, which concurs with the literature (He et al., 2018; Huang et al., 2016; Matamoros, Puigagut, Garcia, & Bayona, 2007). These findings suggest that additional treatment may be required, particularly if reused greywater is utilized in agricultural applications. Biochar and membrane bioreactors have both been indicated as potentially useful for the removal of contaminants of emerging concern. Thus, incorporating these approaches into off-grid greywater treatment may be a viable next step (Meuler, Paris, & Hackner, 2008; Moges, Eregno, & Heistad, 2015).

Additionally, other research groups have noted that different filtration substrates, flow rates, and plant species can improve the removal of antibiotics in greywater treatment systems (Chen, Wei, et al., 2016; Chen, Ying, et al., 2016) For example, Chen et al. (2016) demonstrated improved removal rates of erythromycin with a zeolyte substrate compared to the substrates utilized in the treatment systems tested here or in work by He et al. (2018).

It has been shown that even low concentrations of antibiotics in wastewater effluent can select for antibiotic resistance in natural systems, and pharmaceutical residues can accumulate in the edible portions of plants (Alistair et al., 2012; Gullberg et al., 2011; Martinez, 2009; Paltiel et al., 2016). Given these findings, additional research is warranted on the impact of sub-therapeutic levels of antibiotics within off-grid greywater treatment systems on the selection of resistant bacteria in soil and on produce that comes in contact with reused greywater.

Furthermore, a community- and farmer-based approach is critical for future research. Prior research in the West Bank has suggested that community capacity and knowledge of proper greywater system maintenance is integral to proper functionality of the systems, and research globally has noted that personal behaviors can drive the presence of contaminants of emerging concern in greywater (Halasah, 2017; Chung & Brooks, 2019). Thus, future work should involve the community to assess their capacity to maintain the systems as well as behaviors which may drive introduction of contaminants of emerging concern. If potential causes of variation in contaminant levels can be identified, then "best practices" could be developed to guide greywater system owners.

Limitations

While longitudinal data were collected during the course of our study, long-term data were only collected on three systems. Additionally, these data are limited in geographic scope to the West Bank, Palestinian Territories. Our small sample size and geographic constraints reduce the capacity to draw conclusions about the overall effectiveness of greywater treatment systems. Larger sample sizes, and sampling conducted over a longer time period could allow researchers to evaluate the impact of variable weather conditions on the efficacy of greywater treatment systems, as temperature fluctuations have been shown to affect constructed wetland function (Breitholtz et al., 2012). Moreover, future studies would benefit from the inclusion of a survey instrument that could capture specific household dynamics or behaviors that could be driving higher levels of certain contaminants detected in greywater systems.

Conclusions

This work demonstrates that antibiotics and herbicides are potentially widespread in domestic greywater in the West Bank, and treated greywater can contain higher levels of antibiotics and herbicides compared to conventional irrigation water sources, including groundwater stored in surface ponds. Future studies should evaluate the potential soil health and food safety implications regarding the presence of these contaminants in reused greywater, particularly the influence of low-level antibiotics on the selection of antibiotic-resistant bacteria in soil and associated produce. Additional work is warranted concerning how these systems could be improved to remove emerging contaminants more efficiently.

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Chapter 4: Antibiotic-resistant *Escherichia coli* and *Klebsiella* sp. in greywater reuse systems and pond water used for agricultural irrigation in the West Bank, Palestinian Territories

Hillary A. Craddock[a], Suhana Chattopadhyay[a], Younes Rjoub[b], David Rosen[b], Jake Grief[b], Clive Lipchin[b], Emmanuel F. Mongodin[c], Amy R. Sapkota[a]

[a]Maryland Institute for Applied Environmental Health, University of Maryland School of Public Health, College Park, MD, USA

[b]Arava Institute for Environmental Studies, Ketura, Israel

[c]Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA

Abstract

Introduction: Treating and reusing greywater for agricultural irrigation is becoming increasingly prevalent in water-scarce regions such as the Middle East. However, the potential for antibiotic-resistant bacteria to be introduced into food systems or the environment via greywater reuse is a potential area of concern. It is known that off-grid treated greywater often has elevated levels of bacteria, however, little is known regarding the prevalence of antibiotic-resistant bacteria in this water source.

Methods: Samples (n=61) of off-grid, household greywater (influent), treated greywater effluent, and irrigation pond water were collected between October 2017 and June 2018 from four farms in the West Bank, Palestinian Territories. Samples were tested for pH, turbidity, dissolved oxygen (DO), electrical conductivity (EC), and oxidative reduction potential (ORP). Standard membrane filtration was used to enumerate *Escherichia coli*, and isolates (n=88) were purified, confirmed using 16S rRNA sequencing, and subjected to antimicrobial susceptibility testing using microbroth dilution.

Results: The majority of influent (76.5%) and effluent (70.6%) samples had detectable *E. coli*. Turbidity was significantly lower in effluent than influent (*p*-value <0.0001). The pH (*p*-value=0.007), EC (*p*-value=0.02,), DO (*p*-value=0.009), and ORP (*p*-value=0.01) levels were all higher in effluent than influent. The majority of the isolates were confirmed as *Klebsiella* sp. (n=37), followed by *E. coli* (n=32), and the remainder were classified as other (n=19). A higher percentage of effluent isolates were fully susceptible to all tested antibiotics when compared to influent isolates (28.6% vs 18.6%). Resistance was most commonly observed against ampicillin (69.3% of all isolates), trimethoprim-sulfamethoxazole (11.4%), tetracycline (9.1%), and cefazolin (7.9%), and 7.9% of isolates were observed to be multidrug-resistant.

Conclusions: While most chemical water quality parameters were within Israeli and Palestinian wastewater reuse requirements, *E. coli* levels in effluent violated available

standards. These findings suggest that, despite observed decreases in antibiotic resistance between influent and effluent, off-grid greywater treatment systems are still a potential source of both susceptible and antibiotic-resistant bacteria in the agricultural environment.

Introduction

Antibiotic resistance originating from the food supply is a growing public health concern, and risks to food safety posed by pathogens persisting in agricultural irrigation water are increasing (Callahan, Van Kessel, & Micallef, 2019; Liu, Whitehouse, & Li, 2018; US FDA, 2018). Treating and reusing greywater for agricultural irrigation is becoming more prevalent in water-scarce regions such as the Middle East (McIlwaine & Redwood, 2011). However, the potential for antibiotic-resistant bacteria to be introduced into food systems or the environment via greywater reuse is a potential area of concern (Benami, Gillor, & Gross, 2016). Advanced-treated municipal wastewater effluent has been shown to harbor bacteria expressing resistance to multiple antibiotics (Carey et al., 2016; Goldstein et al., 2012; Ottosson, Jarnheimer, Stenström, & Olsen, 2012), and there is evidence that antibiotic resistance genes persisting in treated wastewater can be shared with environmental bacteria via multiple mechanisms (Berglund et al., 2015; Martinez, 2009; Proia et al., 2016; Rizzo et al., 2013). However, there is a dearth of research investigating the prevalence of antibiotic-resistant bacteria in off-grid greywater reuse systems (Troiano et al., 2018).

Wastewater treatment and reuse methods in low-resource settings often focus on greywater (wastewater that does not contain sewage), and treatment modalities include constructed wetlands and upflow gravel filters (Harman et al., 2017; Little, 2014; McIlwaine & Redwood, 2011). Constructed wetlands are typically a series of containers filled with gravel and plants. Removal of bacteria is achieved through exposure to biofilms and ultraviolet light (Little, 2014). Upflow gravel filtration systems combine passive gravel filtration with active pumping of wastewater through anaerobic tanks (Harman et al., 2017). Off-grid systems are an affordable solution to water access challenges in low-resource settings; however, issues relating to foul odors and unacceptably high levels of bacteria have been reported (Alfiya et al., 2013; Arafeh, 2012; Harman et al., 2017; Little, 2014).

Studies conducted in the West Bank, Palestinian Territories have identified high levels of fecal coliforms in constructed wetland system influent, as well as treated effluent (Alfiya et al., 2013; Little, 2014). Culture-based and culture-independent methods have been utilized in Israel to identify bacterial pathogens in both untreated and treated greywater, and, in addition to *E. coli,* other members of the *Enterobacteriaceae* family have been identified in greywater, including *S. enterica*, *Shigella* sp., and *K. pneumoniae* (Benami, Gillor, & Gross, 2016; Gross, Kaplan, & Baker, 2007; Maimon, Friedler, & Gross, 2014; Ronen, Guerrero, & Gross, 2010). Moreover, exposure to antibiotics like triclosan, which have been detected in greywater systems (Donner et al., 2010; Zedek et al., 2015) has been shown to select for antibiotic resistance in environmental isolates (Ledder, Gilbert, Willis, & McBain, 2006; Westfall et al., 2019). Therefore, it is plausible that antibiotics remaining in household greywater may facilitate the selection of antibiotic resistance.

Nevertheless, there are a limited number of studies investigating antibiotic resistance in greywater (Al-Gheethi, Noman, & Ismail, 2015; Troiano et al., 2018). Troiano et al (2018) identified tetracycline-resistant bacteria in Israeli household greywater and also noted a statistically significant increase in tetracycline-resistant bacteria in treated greywater as opposed to raw greywater. Some detected tetracycline-resistant isolates also expressed multidrug-resistance (Toriano et al., 2018). Furthermore, elevated levels of antibiotic-resistant bacterial infections, as well as practices and behaviors that can select for resistant bacterial populations, have been observed in the West Bank (Adwan et al., 2013; Adwan, Jarrar, Abu-Hijleh, Adwan, & Awwad, 2014; Issa & Adwan, 2016; Sawalha, 2008; Sawalha, 2010; Tayem et al., 2013; Zyoud et al., 2015).

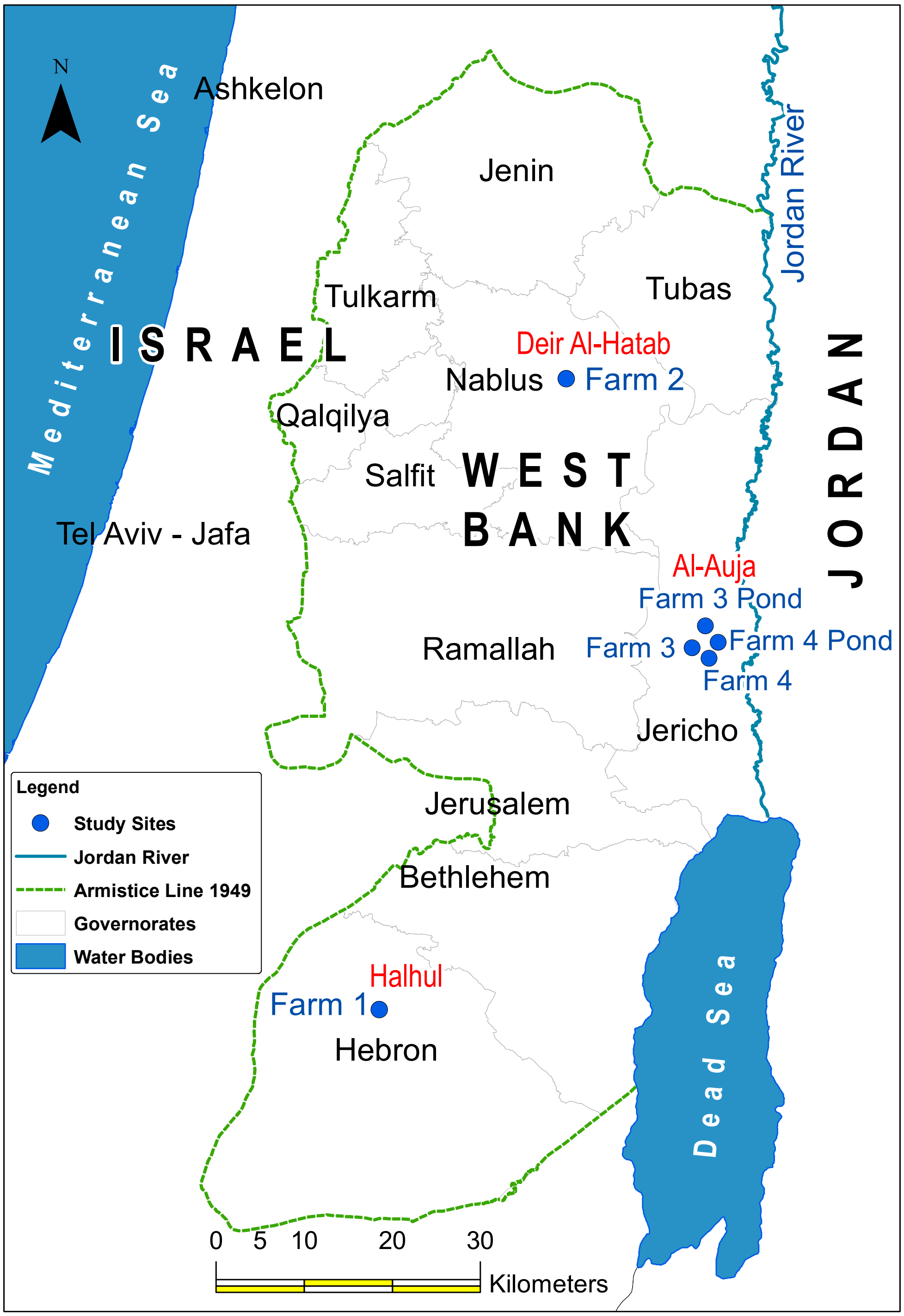
Given that household greywater reuse is a desirable solution for small-scale agricultural irrigation in water-scarce areas like the West Bank (McIlwaine & Redwood, 2011), it is important to further our understanding of the role of greywater reuse systems as a potential source of antibiotic-resistant bacteria. To address this need, we used culture-based methodologies to enumerate *E. coli* and assess phenotypic antibiotic resistance in household greywater influent, treated effluent, and irrigation pond water in the West Bank, Palestinian Territories.

Materials and Methods

Study Sites

Four farms in the West Bank, Palestinian Territories (Figure 1), were included in the study and are described in Craddock et al. (2019). Farm 1, located in the Hebron Governorate, utilizes a constructed wetlands greywater reuse system. Farm 2, located in the Nablus Governorate, also utilizes a constructed wetlands greywater reuse system. Farms 3 and 4 are located in the Jericho Governorate and both utilize upflow gravel filtration greywater reuse systems as well as holding ponds for pumped groundwater. A variety of crops are irrigated with the greywater, including but not limited to: date trees, citrus trees, cucumbers, tomatoes, and zucchini. Drip irrigation is used by all farmers on all crops. Farmers were included in this study based on their prior association with projects launched by members of our team at the Arava Institute for Environmental Studies (Ketura, Israel). Farmers were contacted by phone by a native Arabic speaker to ask if they would be willing to have multiple water samples collected over time from their systems.

Figure 1: Map of study sites, West Bank, Palestinian Territories. Farms 1 and 2 utilize constructed wetlands greywater reuse systems. Farms 3 and 4 utilize upflow gravel filtration greywater reuse systems and holding ponds for pumped groundwater.



Sample Collection

Greywater influent (n=23 samples), greywater effluent (n=24) and irrigation pond water (n=14) grab samples were collected at eight time points between October 2017 and June 2018. Timing of the grab samples was dependent on the availability of farmers as well as political unrest. Additionally, the system in the Nablus Governorate broke down in November and thus only one influent-effluent pair was collected from this system. All samples were collected in sterilized, 1 L Nalgene ® Wide Mouth Bottles (VWR International, Radnor, PA, USA). Samples were transported to the laboratory on ice and stored at 4° C until processing. All samples were tested for pH, electrical conductivity (EC), oxidative reduction potential (ORP), and dissolved oxygen (DO) with Hach HQ30D probes (Loveland, CO, USA). A Sper Scientific Lutu-2016 Lutron Turbidity Meter (Scottsdale, AZ, USA) was used to test for turbidity. A subset of 42 water samples were enumerated for *E. coli* (influent, n=17; effluent n=17; pond n=8).

Membrane Filtration and Enumeration.

Water samples were tested for *E. coli* via standard membrane filtration according to United States Environmental Protection Agency Method 1604 (United States Environmental Protection Agency, 2002). Briefly, 10-fold dilutions of each sample were generated (100, 10-1, 10-2 ,10-3), and 10mL of each dilution (equating to 0.01 mL, 0.1 mL, 1 mL, and 10 mL of each water sample) were vacuum filtered through 0.45 µm, 47 mm mixed cellulose ester membrane filters (Millipore, Billerica, MA, USA). Filters were then aseptically placed on Difco MI Agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and incubated at 35°C for 24 hr (United States Environmental Protection Agency, 2002). After 24 hr, blue or indigo colonies observed under ambient light were counted and considered to be presumptive *E. coli.* For quality control and quality assurance throughout the membrane filtration process, phosphate-buffered saline was used as a negative control.

From each series of dilution plates, up to 14 blue/indigo colonies (presumptive *E. coli*) were picked, streaked on MacConkey agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and incubated at 37°C for 24 hr. Individual colonies from the MacConkey plates were picked and archived in Brucella broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with 15% glycerol at -20°C. Archived isolates (total n=88, including 59 from influent, 21 from effluent, and 8 from pond water) were shipped from Israel to the US on dry ice, and then stored at –80°C.

DNA Extraction and Sequencing

Archived isolates were streaked onto MacConkey agar plates and incubated at 37°C for 24 hr. For each isolate, a quick lysis method was utilized to extract DNA from a single colony. Briefly, a single colony was added to 100 uL of a 7.5% Chelex 100 resin solution (Bio-Rad, Hercules, CA) and heated for 10 min at 105°C in a heat block (Micallef, Callahan, & Pagadala, 2016).

16S rRNA sequencing was conducted on the single isolates described above to confirm their taxonomies. The V3V4 hypervariable region of the 16S rRNA gene was then PCR-amplified, and a dual-indexing strategy for multiplexed sequencing developed at the Institute for Genome Sciences (Baltimore, MD) was then employed, as described previously (Fadrosh et al., 2014). PCR was carried out using 319F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) universal primers (Caporaso et al., 2010; Fadrosh et al., 2014), using thermocycler conditions described previously (Chopyk et al., 2017). After confirming successful amplification via gel electrophoresis, the SequalPrep Normalization Plate kit (Invitrogen Inc., Carlsbad, CA) was then utilized for cleanup and amplicon normalization. The manufacturer's protocol was then followed for 16S rRNA gene sequencing (300-bp paired-end reads) using the Illumina HiSeq 2500 (Illumina, San Diego, CA).

Paired-end 16S rRNA gene sequencing reads were then screened for low quality base pairs and short length, assembled using PANDAseq (Masella, Bartram, Truszkowski, Brown, & Neufeld, 2012), demultiplexed, and chimera trimmed using UCHIME (Edgar, Haas, Clemente, Quince, & Knight, 2011). Quality filtered reads were then incorporated into QIIME (v.1.9.1) (Caporaso et al., 2010), and clustered de novo into Operational Taxonomic Units (OTUs) using VSEARCH. Taxonomic assignments were made using the SILVA database v. 132 (Quast et al., 2013), using a 0.97 confidence threshold. Downstream analysis of quality filtered reads was performed with RStudio (v. 1.1.463). The OTU reference sequences and phylogenetic tree file along with the BIOM-formatted OTU table were imported into the R Statistical software using the Phyloseq R package (v. 1.19.1) (McMurdie & Holmes, 2013).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out on confirmed *E. coli* and *Klebsiella* sp. isolates, as well as a group of other *Enterobacteriaceae* that could not be assigned to a genus, using the Sensititre® microbroth dilution system (Trek Diagnostic Systems Inc., Cleveland, OH, USA) in accordance with the manufacturer's instructions. GN4F minimal inhibitory concentration (MIC) plates (Trek Diagnostic Systems Inc., Cleveland, OH, USA), containing the following antibiotics were used: amikacin (AMI), ampicillin (AMP), ampicillin/sulbactam (AS2), aztreonam (AZT), cefazolin (FAZ), cefepime (FEP), ceftazidimine (TAZ), ceftriaxone (AXO), ciprofloxacin (CIP), doripenem (DOR), ertapenem (ETP), gentamicin (GEN), imipenem (IMI), levofloxacin (LEVO), meropenem (MERO), minocycline (MIN), nitrofurantoin (NIT), piperacillin (PIP), piperacillin/tazobactam (PT4), tetracycline (TET), ticarcillin/clauvulanic acid (TIM2), tigecycline (TGC), tobracycline (TOB), and trimethoprim/sulfamethoxazole (SXT). *E. coli* ATCC 25922 was used for quality control. Minimum Inhibitory Concentrations (MICs) were recorded as the lowest concentration of an antimicrobial that completely inhibited bacterial growth. Resistance breakpoints published by the Clinical Laboratory Standards Institute (CLSI) were used to interpret the MICs, with the exception of the tigecycline MICs, which were interpreted according to the manufacturer's guidelines (Clinical Laboratory Standards Institute, 2018; Goldstein et al., 2012; Pfizer Injectables, 2016). Multidrug-resistance (MDR) was defined as resistance to at least one antimicrobial agent in at least three antimicrobial classes (Magiorakos et al., 2012).

Statistical Analysis

To test whether bacterial counts, as well as chemical water quality parameters, differed among influent, effluent, and pond water, repeated measures ANOVA were carried out, and p-values <0.05 were considered statistically significant. To account for skewness, EC, DO, turbidity, and *E. coli* counts were log transformed prior to analysis. *E. coli* counts and chemical water quality parameters were compared to Israeli and Palestinian wastewater reuse standards (Inbar, 2007; Palestine Standards Institute (PSI), 2003), and proposed Israeli greywater standards (Standards Institution of Israel, 2012). Descriptive statistics were used to analyze antibiotic susceptibility patterns. Statistical analyses were carried out in SAS 9.4 (Cary, NC, USA). Plots were created in R (Version 3.5.1, The R Foundation for Statistical Computing, Vienna, Austria), and the map was designed on ArcGIS ArcMap (Version 10.4.1, Esri, Redlands CA, USA).

<u>*Results*</u>

Bacterial Levels

In influent, 76.5% of samples had detectable levels of *E. coli.* The mean was 6.5×10^4 CFUs/100mL (Range: 0 - 7.1×10^5) (Figure 2). In effluent, 70.6% of samples had detectable levels of *E. coli.* The mean was 7.4×10^4 CFUs/100mL (Range: 0 - 1.1×10^6) (Figure 2). 100% of pond samples had detectable levels of *E. coli*. However, the mean was much lower than that of influent and effluent samples: 820 CFUs/100mL (Range: 10 – 2.9×10^3) (Figure 2). Nevertheless, no statistically significant difference in *E. coli* levels was observed between influent and effluent (*p*-value=0.38) or between effluent and pond water (*p*-value=0.86).

Chemical Water Quality Parameters

Chemical water quality values are presented in Figure 3. Regarding pH, the mean observed value was 6.9 for influent (range: 5.2 - 8.6), 7.6 for effluent (range: 6.6-8.7), and 7.9 for pond water (range: 7.5 - 8.5). For turbidity, the mean observed value was 340.6 NTUs for influent (range: 32.4-1194.3 NTUs), 29.3 NTUs for effluent (range: 2.8 - 128.0 NTUs), and 10.9 NTUs for pond water (range: 1.4 - 60.3 NTUs). For DO, the mean observed value for influent was 1.5 mg/L (range: 0.1 - 6.3 mg/L), 4.5 mg/L for effluent (range: 0.1 - 9.0 mg/L), and 10.5 mg/L for pond water (range: 7.8 - 13.6 mg/L). For EC, the mean observed value was 1238.4 S/m in influent (range: 288.0 - 3153.3 S/m), 1277.0 S/m in effluent (range: 10.8 - 2560.0 S/m), and 1.1 $x10^4$ S/m in pond water (range: 3.6 - 2.6 $x10^4$ S/m). Regarding ORP, the mean observed value was -41.2 mV in influent (range: -278.2 - 157.5 mV), 32.1 mV in effluent (range: -145.2 - 157.4 mV), and 56.6 mV in pond water (Range: -0.6 - 159.5 mV).

When comparing chemical water quality values of influent with effluent, turbidity was significantly lower in effluent than influent (p-value<0.0001). The pH (*p*-value=0.007), EC (*p*-value=0.02), DO (*p*-value=0.009), and ORP (*p*-value=0.01) were all higher in effluent than influent. DO was significantly higher in pond water than in effluent (*p*-value=0.02). The pH (*p*-value=0.2), electrical conductivity (*p*-value=0.06), and ORP (*p*-value=0.1) of pond water was higher than that of effluent, and turbidity of pond water was lower than the turbidity of effluent (*p*-value=0.2), however none of those differences were statistically significant.

Phenotypic Antibiotic Resistance

Resistance to multiple antibiotics was observed among isolates recovered from influent, effluent, and pond water samples (Figure 4). Intermediate susceptibility, or incomplete susceptibility to antimicrobial agents, was also observed against several antibiotics (Clinical Laboratory Standards Institute, 2018; Table 1). Across all influent, effluent and pond samples, resistance was most commonly observed against ampicillin (69.3% of all isolates), trimethoprim-sulfamethoxazole (11.4%), tetracycline (9.1%), and cefazolin (7.9%). All tested isolates were susceptible to amikacin, ertapenem, meropenem, and tigecycline. Intermediate susceptibility, but not resistance, was observed for ceftazidimine, imipenem, piperacillin/tazobactam, and ticarcillin/clauvulanic acid.

As seen in Figure 4, *Klebsiella* sp. isolates exhibited resistance to fewer antibiotics than *E. coli* isolates. Additionally, influent isolates exhibited resistance to more antibiotics than effluent isolates. Both influent and effluent isolates exhibited resistance to more antibiotics than those recovered from pond water (Figure 4).

Overall, multiple susceptibility patterns were observed, as seen in Table 2. Some isolates were susceptible to all tested antibiotics; 22.7% of all isolates, 18.6% of influent isolates and 28.6% of effluent isolates were fully susceptible. A large percentage of both influent (61.0%) and effluent (33.3%) isolates were resistant to ampicillin only. Multidrug-resistance was also observed in all water types (Table 2); 7.9% of isolates exhibited MDR.

Figure 2: *E. coli* detected in household greywater influent (n=17), treated greywater effluent (n=17), and irrigation pond water (n=8) in the West Bank, Palestinian Territories. Solid line is maximum CFUs/100mL for high-quality Palestinian wastewater reuse (100 CFUs/100mL), dashed black line is maximum CFUs/100mL for good-, medium-, and low-quality Palestinian reuse (1,000 CFUs/100mL).

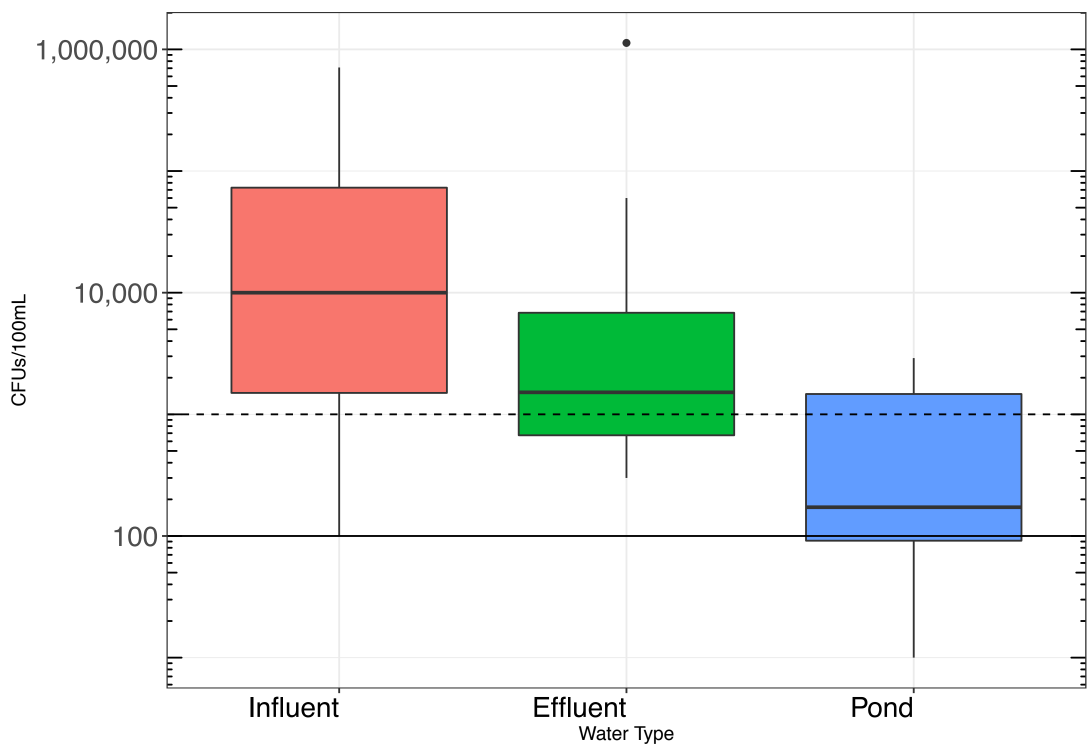
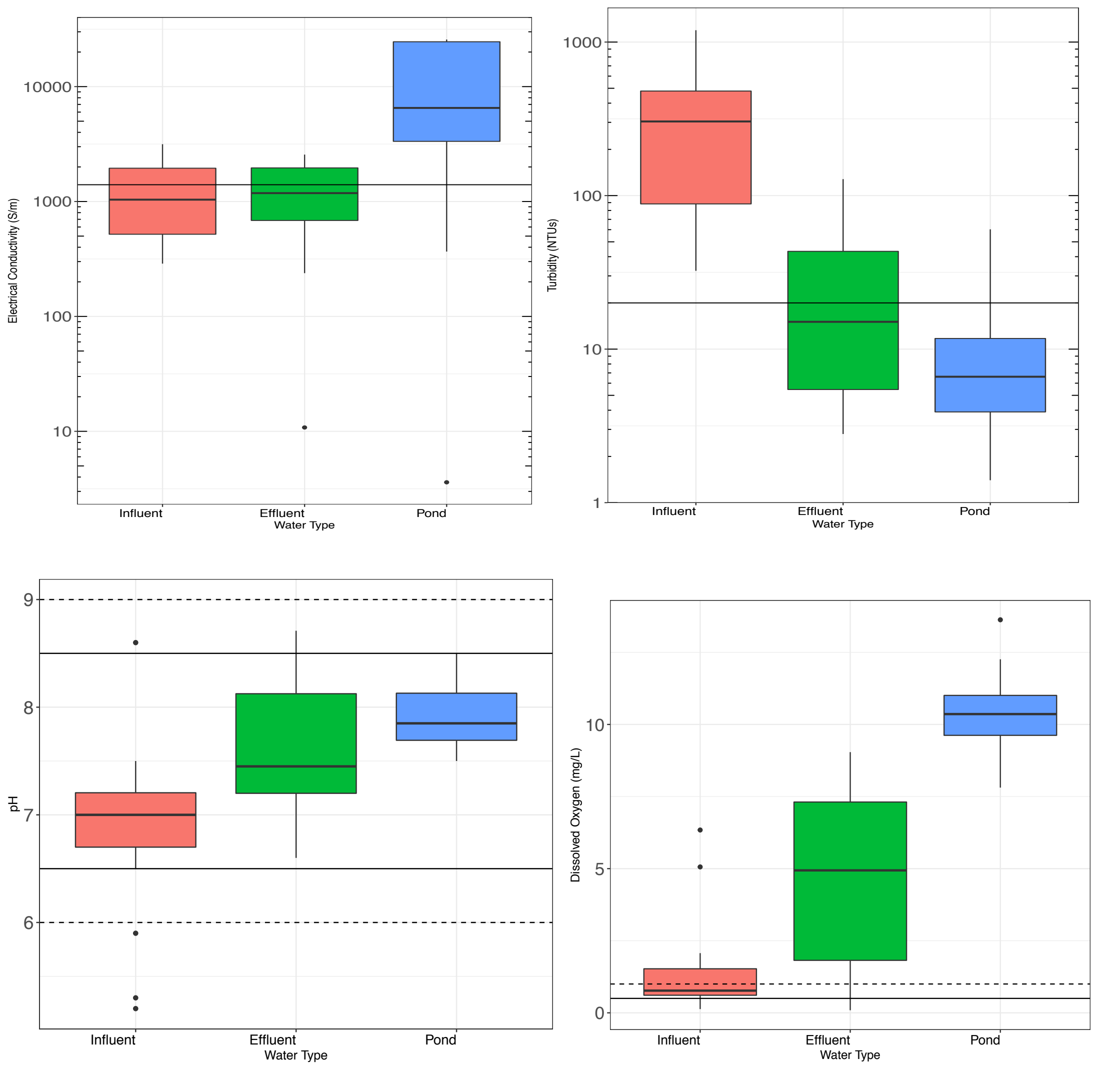
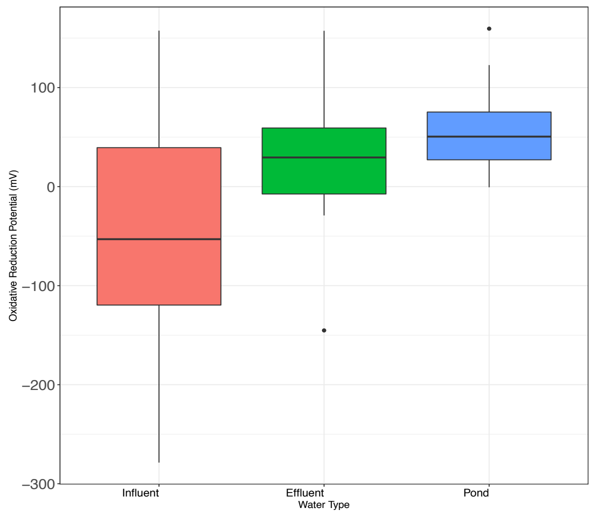


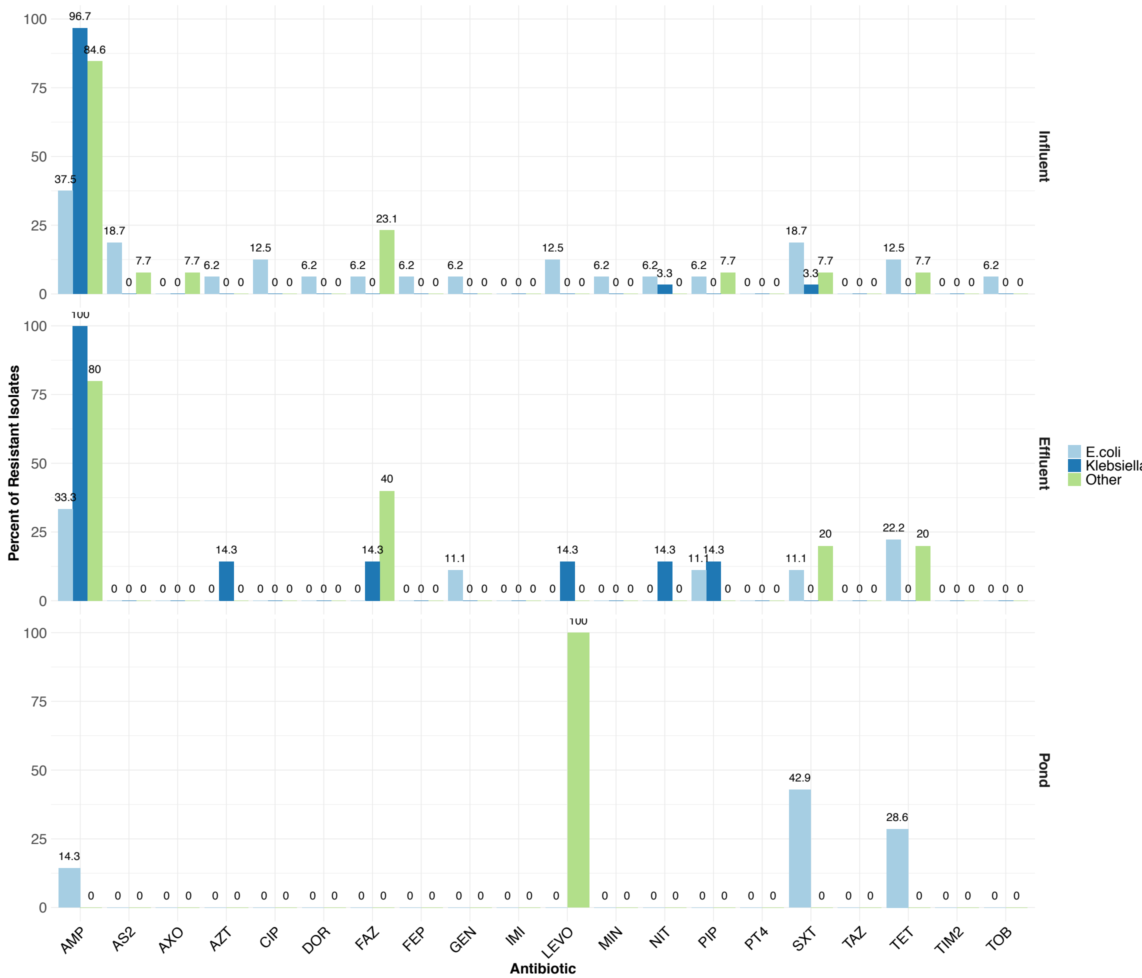
Figure 3: Chemical water quality parameters of household greywater influent (n=23), treated greywater effluent (n=24) and irrigation pond water (n=14) in the West Bank, Palestinian Territories. Top to bottom, left to right: electrical conductivity (S/m), turbidity (NTUs), pH, dissolved oxygen (mg/L), oxidative reduction potential (mV). Solid lines represent Israeli water reuse standards, and dashed lines represent Palestinian water reuse standards.





Oxidative Reduction Potential (mV)
100
0
−100
−200
−300
Influent
Effluent
Pond
Water Type

Figure 4: Antimicrobial resistance patterns among Enterobacteriaceae isolates from household greywater (n=59), treated greywater effluent (n=21), and agricultural irrigation pond water (n=8) from the West Bank, Palestinian Territories.



* No pond isolates were found to be *Klebsiella* spp.

** AMP = ampicillin, AS2 = ampicillin/sulbactam, AXO = ceftriaxone, AZT = aztreonam, FAZ = cefazolin, FEP = cefepime, CIP = ciprofloxacin, DOR = doripenem, GEN = gentamicin, LEVO = levofloxacin, MERO = meropenem, MIN = minocycline, NIT = nitrofurantoin, PIP = piperacillin, TET = tetracycline, TOB = tobracycline, SXT = trimethoprim/sulfamethoxazole.

Table 1: Percentage of *E. coli, Klebsiella* sp., and other *Enterobacteriaceae* isolates that were susceptible (S), intermediate (I), or resistant (R). Isolates were recovered from household greywater (n=59), treated greywater effluent (n=21), and agricultural irrigation pond water (n=8) from the West Bank, Palestinian Territories.

		E. coli			*Klebsiella* sp.		Other		
		Influent (n=16)	Effluent (n=9)	Pond (n=7)	Influent (n=30)	Effluent (n=7)	Influent (n=13)	Effluent (n=5)	Pond (n=1)
AMP	S	62.5	66.7	85.7	3.3	0	0	20	100
	I	.	.	.	.	.	15.4	0	0
	R	37.5	33.3	14.3	96.7	100	84.6	80	0
AS2	S	68.7	88.9	85.7	96.7	100	84.6	40	100
	I	12.5	11.1	14.3	3.3	0	7.7	60	0
	R	18.7	0	0	.	.	7.7	0	0
FAZ	S	75	66.7	85.7	96.7	85.7	53.8	60	100
	I	18.7	33.3	14.3	3.3	0	23.1	0	0
	R	6.2	0	0	0	14.3	23.1	40	0
PIP	S	87.5	88.9	85.7	96.7	85.7	84.6	80	100
	I	6.2	0	14.3	3.3	0	7.7	20	0
	R	6.2	11.1	0	0	14.3	7.7	0	0
PT4	S	93.7	100	100	100	100	92.3	100	100
	I	6.2	0	0	.	.	7.7	0	0
	R	.	.	.	.	.	.	.	.
TIM2	S	93.7	100	100	100	100	100	100	100
	I	6.2	0	0	.	.	.	.	.
	R	.	.	.	.	.	.	.	.
TET	S	75	77.8	71.4	100	100	92.3	80	100
	I	12.5	0	0	.	.	.	.	.
	R	12.5	22.2	28.6	.	.	7.7	20	0
MIN	S	87.5	100	100	100	100	100	100	100
	I	6.2	0	0	.	.	.	.	.
	R	6.2	0	0	.	.	.	.	.
GEN	S	93.7	88.9	100	100	100	100	100	100
	I	.	.	.	.	.	.	.	.
	R	6.2	11.1	0	.	.	.	.	.
TOB	S	93.7	88.9	100	100	100	100	100	100
	I	0	11.1	0	.	.	.	.	.
	R	6.2	0	0	.	.	.	.	.
FEP	S	93.7	100	100	100	100	100	100	100
	I	.	.	.	.	.	.	.	.
	R	6.2	0	0	.	.	.	.	.
AXO	S	93.7	100	100	96.7	100	92.3	100	100
	I	6.2	0	0	3.3	0	.	.	.
	R	.	.	.	.	.	7.7	0	0
TAZ	S	93.7	100	100	100	85.7	100	100	100
	I	6.2	0	0	0	14.3	.	.	.
	R	.	.	.	.	.	.	.	.
CIP	S	87.5	100	100	100	100	100	100	100
	I	.	.	.	.	.	.	.	.
	R	12.5	0	0	.	.	.	.	.
LEVO	S	87.5	100	100	100	85.7	100	100	0
	I	.	.	.	.	.	.	.	.
	R	12.5	0	0	0	14.3	0	0	100
AZT	S	93.7	100	100	100	85.7	100	100	100
	I	.	.	.	.	.	.	.	.
	R	6.2	0	0	0	14.3	.	.	.
SXT	S	81.2	88.9	57.1	96.7	100	92.3	80	100
	I	.	.	.	.	.	.	.	.
	R	18.7	11.1	42.9	3.3	0	7.7	20	0
DOR	S	93.7	100	100	100	100	100	100	100
	I	.	.	.	.	.	.	.	.
	R	6.2	0	0	.	.	.	.	.
IMI	S	93.7	100	100	100	100	100	100	100
	I	6.2	0	0	.	.	.	.	.
	R	.	.	.	.	.	.	.	.
NIT	S	81.2	88.9	100	80	57.1	69.2	80	100
	I	12.5	11.1	0	16.7	28.6	30.8	20	0
	R	6.2	0	0	3.3	14.3	.	.	.

* No pond isolates were found to be *Klebsiella* sp.

** AMP = ampicillin, AS2 = ampicillin/sulbactam, AXO = ceftriaxone, AZT = aztreonam, FAZ = cefazolin, FEP = cefepime, CIP = ciprofloxacin, DOR = doripenem, ETP = ertapenem, GEN = gentamicin, IMI = imipenem, LEVO = levofloxacin, MERO = meropenem, MIN = minocycline, NIT = nitrofurantoin, PIP = piperacillin, PT4 = piperacillin/tazobactam, TAZ = ceftazidimine, TET = tetracycline, TIM2 = ticarcillin/clauvulanic acid, TOB = tobracycline, SXT = trimethoprim/sulfamethoxazole.

*** Resistance or intermediate susceptibility was not observed against amikacin, ertapenem, meropenem, and tigecycline.

Table 2: Antibiotic resistance patterns among *E. coli, Klebsiella* sp., and other *Enterobacteriaceae* isolates recovered from household greywater (n=59), treated greywater effluent (n=21), and agricultural irrigation pond water (n=8) from the West Bank, Palestinian Territories.

	Influent	**Effluent**	**Pond**
No resistance	11 (18.6%)	6 (28.6%)	3 (37.5%)
AMP only	36 (61.0%)	7 (33.3%)	0
SXT only	0	0	2 (25.0%)
TET only	1 (1.7%)	0	1 (12.5%)
LEVO only	0	0	1 (12.5%)
AMP-FAZ	2 (3.4%)	2 (9.5%)	0
AMP-AS2	1 (1.7%)	0	0
AMP-SXT	1 (1.7%)	0	0
AMP-NIT	1 (1.7%)	1 (4.8%)	0
NIT-TET	0	1 (4.8%)	0
AMP-AS2-SXT	2 (3.4%)	0	0
AMP-AZT-FAZ	0	1 (4.8%)	0
AMP-LEVO-PIP	0	1 (4.8%)	0
MDR isolates	**4 (6.8%)**	**2 (9.5%)**	**1 (12.5%)**
AMP-SXT-TET	0	1 (4.8%)	1 (12.5%)
LEVO-AZT-CIP	1 (1.7%)	0	0
AMP-FEP-DOR-NIT	1 (1.7%)	0	0
AMP-GEN-PIP-SXT-TET	0	1 (4.8%)	0
AMP-TET-SXT-PIP-FAZ-AXO	1 (1.7%)	0	0
AMP-LEVO-NIT-TET-MIN-SXT-PIP-GEN-FAZ-TOB-AS2-AMP-CIP	1 (1.7%)	0	0

*AMP = ampicillin, AS2 = ampicillin/sulbactam, FAZ = cefazolin, CIP = ciprofloxacin, GEN = gentamicin, LEVO = levofloxacin, MIN = minocycline, TET = tetracycline, SXT = trimethoprim/sulfamethoxazole

Discussion

In this study, we demonstrated that greywater reuse has the potential to introduce more bacteria as well as more antibiotic-resistant bacteria into the agricultural environment when compared to Palestinian farmers' normal source of irrigation water. Both median and mean *E. coli* levels in effluent were in violation of the Palestinian requirements for wastewater reuse. While these systems do reduce bacterial levels, this reduction is insufficient to deal with the bacterial load going into the systems. Studies of similar off-grid greywater treatment systems in Israel have also found high levels of bacteria, and the studies also noted that treatment was insufficient to reduce bacteria to acceptable levels (Gross et al., 2007; Ronen et al., 2010).

Regarding other water quality parameters, efforts need to be made to reduce turbidity, as this was the only parameter for which both the mean and the median of the effluent data did not meet the proposed Israeli standard. It also bears noting that there was no significant difference between most chemical water quality parameters in effluent and pond water. EC was observed to increase throughout the treatment process, and this should be an area of concern as farmers are often dealing with saline groundwater (Da'as & Walraevens, 2010).

Phenotypic Antibiotic Resistance in Greywater, Effluent, and Pond Water

Given the wide variety of resistance to different antibiotics observed in different West Bank studies of human infections, identifying resistant bacteria in greywater as well as treated effluent was not surprising (Adwan et al., 2014, 2013;

Issa & Adwan, 2016; Sawalha, 2009). The only thing which could be considered unexpected when comparing our findings to previous studies of antibiotic-resistant infections in the West Bank was our low observed rates of resistance to ciprofloxacin.

When comparing antimicrobial susceptibility patterns observed among influent, effluent, and pond water isolates there is a notable difference, namely that isolates were resistant to a greater number of antibiotics in influent and effluent water than in pond water. However, it is important to note that this may be an artifact of low isolate numbers. To the author's knowledge this is the first paper to provide preliminary data comparing reused greywater with farmers' normal source of irrigation water. Our findings suggest that even treated greywater may pose an increased risk of introducing antibiotic resistance into the agricultural environment.

Implications for development and future research

While this and other research studies suggest that greywater treatment systems can reduce bacterial loads, the loads going into the systems are far too high. Ronen et al. (2010) found that this was primarily attributed to kitchen greywater; however, given the high levels of water scarcity in this region, future research should focus on addressing the bacterial loads of kitchen greywater, not simply excluding it. Further research should focus on incorporating a disinfection or polishing step for these systems.

Furthermore, a larger-scale study should be carried out to assess antibiotic resistance, both phenotypic and genotypic, in greywater, treated effluent, and the source of water the farmer would use if the greywater was not available. Ultimately, this comparison with what the farmer would be using if the greywater were not available is critical in assessing if the greywater is more problematic than what would be used otherwise. It also bears noting that the two sampled ponds were examples of highly saline groundwater due to groundwater salinity issues in the region (Rosenthal, Vinokurov, Ronen, Magaritz, & Moshkovitz, 1992; Tal, Weinstein, Yechieli, & Borisover, 2017). Therefore, future efforts should be made to sample a wider variety of irrigation water sources in the West Bank.

Future studies should also delve into factors that influence antibiotic and bacterial loading levels as well as the presence of antibiotic resistance in households contributing to these systems. These data could be used to develop best management practices for the owners of these systems. Another important issue to assess is whether the high bacterial levels detected in greywater reuse systems have measurable impacts on human health. An epidemiological study carried out among household greywater users in Israel noted no relationship between greywater reuse and gastrointestinal illness; however, less than half of the population was reusing greywater for edible crops, and none of the edible crops were being eaten raw (Busgang, Friedler, Ovadia, & Gross, 2015). Thus, further epidemiological investigation, especially considering that some crops produced in our study are intended to be eaten raw, is warranted.

Limitations

Our primary limitation is a small sample size, both in terms of number of systems sampled as well as number of isolates tested for antibiotic resistance. Given that grab samples were taken on the same day, these are also not true paired influent-effluent pairs. By taking multiple samples over time and looking at the overall trends, we sought to address this challenge, but it is still a concern.

Conclusions

This work demonstrates that in addition to concerns regarding high levels of bacteria, antibiotic-resistant bacteria are potentially widespread in domestic greywater in the West Bank. Furthermore, treated greywater can contain higher levels of antibiotic-resistant bacteria than conventional irrigation water sources, including groundwater stored in surface ponds. Future studies should evaluate the potential soil health and food safety implications regarding the presence of these contaminants in reused greywater, particularly via the integration of culture-independent research methodologies. Additional work is warranted concerning how these systems could be improved to remove emerging contaminants more efficiently.

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Chapter 5: Perceptions on the use of recycled water for produce irrigation and household tasks: A comparison between Israeli and Palestinian consumers

Hillary A. Craddock[a], Kristal Jones[b], Younes Rjoub[c], Clive Lipchin[c], Amy R. Sapkota[a]

[a]Maryland Institute for Applied Environmental Health, University of Maryland School of Public Health, College Park, Maryland, USA

[b]National Socio-Environmental Synthesis Center, University of Maryland, Annapolis, Maryland, USA;

[c]Arava Institute for Environmental Studies, Ketura, Israel

Abstract

Introduction: Water scarcity has resulted in extensive wastewater recycling for agricultural irrigation in both Israel and the Palestinian Territories. However, minimal data have been collected regarding perceptions about wastewater recycling between these populations. While geographically and economically close, these two populations differ in terms of governance, income levels, and access to technology for wastewater recycling.

Methods: To address this data gap, a survey was administered among a convenience sample of subjects (n=236) recruited in both Eilat, Israel and Bethlehem, West Bank, from May to June 2018. The survey included 54 questions addressing food purchasing habits; knowledge of water sources, scarcity, and recycled water; willingness to use recycled water for produce irrigation and household tasks; and demographics. Descriptive statistics, Chi-squared analysis, and Fisher's exact tests were used to analyze the data.

Results: The two surveyed populations differed across all sociodemographic factors, including age, gender, religion, education, and household income. Perceptions of regional water scarcity and water contamination varied between the two populations; Palestinian respondents were more likely than Israeli respondents to agree that their region had recently experienced drought or water contamination. More Palestinian respondents than Israeli respondents reported using recycled water both from home and from WWTPs in the past. Less than half of respondents from both populations were familiar with the technologies used to treat recycled water. Israeli willingness to use recycled water for various purposes ranged from 8.3% - 55.1%, and more than half of Israeli respondents were willing to serve both raw and cooked produce irrigated with recycled water. Willingness to use recycled water ranged from 28.9% - 41.5% among the Palestinian respondents, and Palestinian respondents were more willing to engage in high-contact uses (i.e. drinking and cooking) than Israeli respondents. Among the Israeli respondents, experience or familiarity with

wastewater recycling and water contamination experience were frequently significantly associated with willingness to use recycled water. In contrast, among Palestinian respondents, personal water contamination experience, home water safety testing, and trust in authorities to monitor recycled wastewater reuse were frequently significantly associated with willingness to use recycled water.

Conclusions: Our findings suggest that both Israeli and Palestinian populations are willing to use recycled water in a variety of ways, although willingness varies by the specific type of reuse application and by population. Given the likely increasing water stress in both Israel and the Palestinian Territories, as well as the continued evolution of wastewater treatment technologies, it is important to identify effective and appropriate outreach and communication strategies to enable successful water reuse.

Terminology note: As “recycled water” was the term used throughout the survey, this language will be used in this dissertation aim as opposed to reused wastewater.

Introduction

The use of recycled water (municipal wastewater that has been treated for reuse in agricultural and domestic activities) can elicit an emotionally-charged response in some communities (Dolnicar, Hurlimann, & Grün, 2011; Hurlimann & Dolnicar, 2010; Schwartz, 2015). As such, extensive stakeholder engagement is often critical for the success of new wastewater recycling projects (Morgan & Grant-Smith, 2015; Rozin, Haddad, Nemeroff, & Slovic, 2015). Specifically, understanding community knowledge levels and concerns and responding with appropriate outreach and engagement can be important for new wastewater recycling endeavors (Dolnicar, Hurlimann, & Nghiem, 2010). However, in some highly water stressed regions, stakeholder engagement has not been fully developed despite ongoing wastewater recycling (Lipchin, 2006; Tal, 2006). This lack of stakeholder engagement potentially threatens the success of wastewater recycling projects and filling this data gap will also contribute to outreach and education efforts that fill an actual need within the studied communities.

In the Middle East, for instance, water scarcity has necessitated wastewater recycling for agricultural irrigation in both Israel and the Palestinian Territories (Friedler, 2001; McNeill, Almasri, & Mizyed, 2009). In Israel, about 90% of municipal wastewater is recycled, and of that more than 80% is used for agricultural irrigation (Berman et al., 2017; Dotan et al., 2016). About half of all irrigation water used in Israel is recycled municipal wastewater (Tal, 2016). In contrast, the West Bank recycles less than ten percent of its municipal wastewater (Dotan et al., 2016; McIlwaine & Redwood, 2011; Mizyed, 2013), and use of untreated wastewater for irrigation is a known practice (Bieler, 2016; Ezery, 2016). Despite this ongoing and extensive use of recycled water, as well as significant transboundary trade (Bank of Israel, 2014; Venghaus, 2017), minimal data have been collected regarding knowledge and perceptions of wastewater recycling practices among Israelis and Palestinians.

One Israeli survey assessed consumer acceptance of various uses of recycled water, including but not limited to clothes washing (45% acceptance), vegetable irrigation (48%), and orchard irrigation (53%) (Friedler, 2008). In contrast, a moderate number of studies have examined the public's opinions concerning the use of recycled water on food crops in the West Bank. Across studies assessing consumer perceptions in the West Bank, the percentage of respondents that accepted the use of recycled water for agricultural irrigation ranged from 25 – 90% (Abu Madi, Mimi, & Abu-Rmeileh, 2008; Al-Kharouf, Al-Khatib, & Shaheen, 2008; Al-Sa'ed & Mubarak, 2006; Faruqui, Biswas, & Bino, 2000; Ghanem, Isayed, & Abu-Madi, 2010). One study that compared cooked and raw produce irrigated with recycled water found that the cooked produce was more acceptable to consumers (47% acceptance vs 32%, respectively) (Ghanem, Isayed, & Abu-Madi, 2010). Degree of contact is hypothesized to be an important factor in recycled water acceptance (Hartley, 2006; Rock, Solop, & Gerrity, 2012). While Friedler (2008) investigated recycling applications involving varying degrees of contact (i.e. low direct contact options like toilet flushing, compared to high direct contact options like clothes washing), there are, to the author's knowledge, no surveys in the West Bank that have investigated water recycling applications other than those pertaining to agricultural irrigation.

These findings, coupled with the lack of studies using the same survey instrument to compare Israeli and Palestinian populations, demonstrate a need for more in-depth research on consumer willingness to use recycled water. The similarity of climate and groundwater challenges between Israel and the Palestinian Territories, as well as the stark discrepancies in governance structures, income, education, and religion across these populations, makes this an important comparison with implications for both development projects and future management of water resources in the Middle East. To address these issues, we administered a survey to evaluate perceptions regarding the use of recycled water for produce irrigation and household tasks among Israeli and Palestinian respondents.

Materials and Methods

Study Sites and Population

This study included two sites: Eilat, Israel and Bethlehem, West Bank, Palestinian Territories (Figure 1). Eilat is a city of 50,072 in the south of Israel, with a mostly Hebrew-speaking, Jewish population (Israeli Central Bureau of Statistics, 2012) (Israeli Central Bureau of Statistics, 2019). Bethlehem is a city of 28,248 in the south-central part of the West Bank, with a primarily Arabic-speaking, Muslim population (Palestinian Central Bureau of Statistics, 2019).

Figure 1: Map of survey study sites in Eilat, Israel and Bethlehem, West Bank, Palestinian Territories.

Recruitment and Survey Administration

We recruited a convenience sample of subjects from public locations in both study sites in May and June 2018. We provided a small incentive, and subjects either completed a paper-based survey or an online version of the same survey. A secure link to the online survey was also shared via social media, specifically through Southern Israel- and West Bank-specific Facebook Groups, in order to reach more residents of each area. The online survey was available from May to November 2018. The survey and study were reviewed and approved by the University of Maryland, Institutional Review Board (IRB).

Survey Instrument

The survey included 54 multiple-choice and open-ended questions. Questions covered food purchasing habits; knowledge of water sources and scarcity in their region; knowledge about recycled water; willingness to use recycled water for produce irrigation and household tasks; and demographics. The study was initially designed in English and then translated into Hebrew and Arabic. During translation, the framing or explanation of some survey questions was adapted to the cultural context of Israel and the Palestinian Territories. The Israeli survey was available in both Hebrew and English to more fully capture the Israeli population. The online survey was developed and stored on the survey platform Qualtrics (Qualtrics, Provo, Utah, USA), and paper-based surveys were entered into the Qualtrics platform after collection.

Statistical Analysis

Analysis was conducted with descriptive statistics, Chi-squared analysis, and Fisher's exact tests. Fisher's exact tests were used when required by sample size. We recategorized responses from a five-point likert scale (Strongly agree, Agree, Neutral, Disagree, Strongly disagree) into two levels (Agree, Did not agree) based on the assumption that there is little meaningful difference between the two positive values (Agree and Strongly agree) and the three neutral or negative values (Neutral, Disagree, and Strongly disagree). Furthermore, our primary outcome of interest was agreement with using recycled wastewater, and recategorization allowed the analysis to focus on factors associated with agreement. To test for within-group significance among demographic variables, cell residuals were calculated as a post-hoc analysis and a Bonferroni adjustment was utilized to adjust the *p*-value required for significance (Shan & Gerstenberger, 2017).

P-values <0.05 were considered statistically significant. Data analysis was conducted with SAS 9.4 (Cary, NC). Figures were created in R (Version 3.5.1, The R Foundation for Statistical Computing, Vienna, Austria), and the map was designed on ArcGIS ArcMap (Version 10.4.1, Esri, Redlands CA, USA).

Results

Population demographics

The study included a total of 236 survey respondents: 127 individuals were surveyed from Israel and 109 from the Palestinian Territories. 64.0% of respondents chose to respond via the paper-based survey. As shown in Table 1, the Israeli and Palestinian respondents differed significantly based on age distribution, gender, religion, education, and income. The Israeli respondents were slightly more than half female (58.7%), whereas the Palestinian respondents skewed male (71.3%). The Israeli respondents were majority Jewish (80.5%), and the Palestinian respondents were majority Muslim (92.6%). A greater proportion of Israelis reported no religion, and there was no significant difference between the two populations regarding respondents reporting Christian or "other" religious affiliation. The Palestinian respondents were generally younger (77.3% were 30 or younger) than the Israeli respondents (45.3% were 30 or younger). The statistical significance of this difference was driven by three age categories: a greater proportion of Palestinian respondents than Israeli respondents were in the 21-30 age category, and a greater proportion of Israeli respondents than Palestinian respondents were in the 51-60 and 60+ age categories.

Palestinian respondents generally reported lower household income than Israeli respondents. More Palestinian than Israeli respondents reported that their household income was <3,000 NIS (<830 USD) and 3,000-5,000 NIS (830 – 1,400 USD), and more Israeli than Palestinian respondents reported that their household income was 5,001 – 10,000 NIS (1,400 – 2,700 USD), 10,001 – 20,000 NIS (2,700 – 5,500 USD), and >20,000 NIS (> 5,500 USD). Israeli respondents generally reported higher levels of educational attainment than Palestinian respondents. More Palestinian than Israeli respondents reported having less than a high school education, and more Israeli than Palestinian respondents reported having a graduate degree. There was no significant difference between the proportions of respondents reporting a high school level education, some college, or a bachelor's degree.

72.6% of Israeli respondents who answered the question regarding ethnicity reported having Eastern European, Ashkenazim, or white ethnicity, while the remaining 27.4% reported whole or part Mizrahim and/or Sephardim ethnicity. 37.1% of Palestinian respondents reported Bedouin heritage, whereas 62.9% did not have Bedouin heritage or weren't sure.

Comparison between Israeli and Palestinian responses

As seen in Figures 2, 3, and 4, there were significant differences between Israeli (IL) and Palestinian (PA) respondents in regard to food and water perceptions, wastewater experience, wastewater perceptions, and willingness to use recycled water.

As seen in Figure 2, More Israeli respondents than Palestinian respondents agreed that the food they wanted to buy was available and affordable to them (IL: 65.0%, PA: 39.1%, p-value=0.0002). Less than half of both populations agreed that they buy food that has been produced in ways that are sustainable for the environment (IL: 36.5%, PA: 47.1%, p-value=0.1). Palestinian respondents were more likely than Israeli respondents to agree that their region had recently experienced a lack of water or drought (IL: 36.7%, PA: 50.6%, p-value= 0.05) and water contamination (IL: 17.9%, PA: 42.9%, p-value=0.0002). They were also more likely than Israeli respondents to agree that they had personally experienced the effects of water contamination (e.g. water made someone sick or they had been exposed to warnings not to drink or bathe in tap water) (IL: 12.6%, PA: 36.0%, *p*-value=0.0002). Less than half of both populations agreed that they had personally experienced the effects of water scarcity or drought (e.g. water use restrictions) (IL: 32.8%, PA: 31.8%, *p*-value=0.9). Palestinian respondents were more likely than Israelis to agree that they had tested the safety of the water in their home (e.g. lead test) (IL: 24.8%, PA: 47.4%, *p*-value=0.002).

As seen in Figure 3, regarding wastewater experience less than half of both populations agreed that they were familiar with the technologies used to treat wastewater (IL: 38.9%, PA: 44.0%, *p*-value=0.7), that they had used recycled water from a wastewater treatment plant (WWTP) (IL: 23.8%, PA: 27.7% , *p*-value=0.7), or that they had used water (e.g. rain water or greywater) collected at their homes (IL: 30.4%, PA: 42.5%, *p*-value=0.06). Palestinian respondents were more likely than Israeli respondents to agree that recycled water was more likely to pollute the soil (IL: 19.8%, PA: 52.4%, *p*-value<0.0001) than conventional irrigation water. Less than half of both populations agreed that recycled water has a higher risk of contamination by bacteria or other pathogens (IL: 34.6%, PA: 25.3%, *p*-value=0.2) or pharmaceuticals (IL: 35.4%, PA: 43.8%, *p*-value=0.3) than conventional irrigation water. Less than half of both populations agreed that recycled water was more likely to pollute downstream waterways (IL: 25.9%, PA: 33.7%, *p*-value=0.3) than conventional irrigation water.

As seen in Figure 4, the two populations differed significantly on their willingness to use recycled water in various contexts. Israeli respondent willingness ranged from 8.3% - 55.1%, and more than half were willing to serve both raw and cooked produce irrigated with treated wastewater. Willingness among the Palestinian respondents was less varied (28.9% - 41.5%) than willingness among Israeli respondents, and no recycling option garnered agreement from more than half the respondent population.

Less than half of both populations were willing to use recycled water for washing clothes (IL: 31.3%, PA: 33.7%, p-value=0.9) and washing dishes (IL: 19.6%, PA: 28.9%, p-value=0.1). Palestinian respondents were more willing than Israeli respondents to use recycled water for bathing (IL: 18.6%, PA: 41.7%, p-value=0.0005), washing produce (IL: 11.6%, PA: 38.0%, p-value$<$0.0001), cooking (IL: 11.5%, PA: 35.2%, p-value$<$0.0001), and drinking (IL: 8.3%, PA: 30.7%, p-value$<$0.0001). However, Israeli respondents were more willing than Palestinian respondents to serve cooked produce irrigated with recycled water (IL: 55.1%, PA: 37.5%, p-value=0.01) and raw produce irrigated with recycled water (IL: 52.5%, PA: 35.1%, p-value= 0.02).

For counts for each response, see Supplemental Table 1.

Palestinian agreement with various uses of recycled water

The association of different factors with recycled water use agreement are reported in Table 2. Three factors were commonly significant across wastewater recycling options among the Palestinian respondent population: personal water contamination experience, home water safety testing, and trust in authorities to monitor recycled wastewater reuse.

Those who failed to agree that they personally had experienced the impacts of water contamination were more likely to express willingness to use recycled water for washing clothes (*p*-value=0.03), and those who failed to agree that their region had recently experienced water contamination were more likely to express willingness to use recycled water to wash produce (*p*-value=0.03). Those who agreed that they had tested the safety of their water at home were more willing to use recycled water for washing dishes (*p*-value=0.002) and cooking (*p*-value=0.02).

Those who were willing to serve cooked produce irrigated with recycled wastewater were also likely to agree that they trusted their local utility/wastewater treatment system to test and monitor recycled irrigation water (*p*-value=0.01). Those who were willing to serve raw produce irrigated with recycled wastewater were also likely to agree that they trusted the private sector to test and monitor recycled irrigation water (*p*-value=0.03). Those who agreed that they trusted their local utility/wastewater treatment system to test and monitor recycled irrigation water were more willing to use recycled water for bathing (*p*-value=0.04).

For non-statistically significant responses, see Supplemental Table 2.

Israeli agreement with various uses of recycled water

The association of different factors with willingness to use recycled water are reported in Table 3. The following topical areas were commonly associated with willingness to use recycled water among the Israeli respondent population: experience or familiarity with wastewater recycling and water contamination experience.

Those who agreed that they were familiar with the technologies used to treat and recycle wastewater expressed more willingness to use recycled water for washing clothes (*p*-value = 0.006), washing dishes (*p*-value=0.009), cooking (*p*-value=0.0005), washing produce (*p*-value=0.01), and drinking (*p*-value=0.002). Those who agreed that they had used recycled water they had gathered at their home expressed more willingness to serve raw produce irrigated with recycled water (*p*-value=0.007) as well as for using recycled water to wash dishes (*p*-value=0.003) and cook (*p*-value=0.003). Those who agreed that they had used recycled water from a WWTP expressed more willingness to use recycled water for washing clothes (*p*-value=0.001), bathing (*p*-value=0.02), washing dishes (*p*-value=0.01), cooking (*p*-value=0.004), and drinking (*p*-value=0.002).

Those who agreed that they had personally experiencing the effects of water contamination expressed more willingness to serve cooked produce irrigated with recycled water (*p*-value=0.04) as well as to use recycled water to bathe (*p*-value=0.005), cook (*p*-value=0.009), wash produce (*p*-value=0.01), and drink (*p*-value=0.001). Those who agreed that their region of the country had recently experienced water contamination expressed more willingness to use recycled water for washing produce (*p*-value=0.047) and drinking (*p*-value=0.03).

For non-statistically significant responses, see Supplemental Table 3.

Table 1: Demographics of Israeli (n=127) and Palestinian (n=109) respondents of a wastewater recycling survey.

		Israeli	Palestinian	Total	Within-group *p* -value	Overall *p* -value
Age	18-20	20 (15.6)	29 (26.8)	49 (20.8)	0.02	<0.0001
	21-30	38 (29.7)	54 (49.1)	92 (40.0)	0.0007	
	31-40	21 (16.4)	13 (11.8)	34 (14.4)	0.2	
	41-50	7 (5.5)	8 (7.3)	15 (6.3)	0.3	
	51-60	16 (20.3)	2 (1.8)	18 (7.6)	0.001	
	61+	26 (20.3)	2 (1.8)	28 (11.9)	<0.0002	
	Total respondents	128	108	236		
Gender	n, % Female	64 (58.7%)	31 (29.2%)	95 (44.2%)	N/A	<0.0001
	Total respondents	109	106	215		
Religion	Jewish	95 (80.5%)	0 (0%)	95 (42.4%)	<0.0002	<0.0001
	Muslim	0 (0%)	98 (92.4%)	98 (43.7%)	<0.0002	
	Christian	7 (5.9)	6 (5.5%)	13 (5.8%)	0.5	
	None	12 (10.2%)	2 (1.8%)	14 (6.2%)	0.005	
	Other	4 (3.4%)	0 (0%)	4 (1.8%)	0.03	
	Total respondents	118	106	224		
Education	Less than High School	0 (0%)	31 (28.7%)	31 (14.1%)	<0.0002	<0.0001
	High School	39 (34.5)	31 (29.2)	70 (32.0%)	0.2	
	Some College or Bachelor's Degree	37 (32.7)	39 (36.8)	76 (34.7%)	0.3	
	Graduate Degree	37 (32.7)	5 (4.6)	42 (19.2%)	<0.0002	
	Total respondents	113	106	219		
Household Income	<3,000 NIS	1 (1.1%)	33 (42.8)	34 (20.0%)	<0.0002	<0.0001
	3,000 - 5,000 NIS	7 (7.5%)	28 (36.4)	35 (20.6%)	<0.0002	
	5,001 - 10,000 NIS	35 (37.6)	10 (12.66)	45 (26.5%)	<0.0002	
	10,001 - 20,000 NIS	34 (36.6)	2 (2.5)	36 (21.2%)	<0.0002	
	>20,000 NIS	16 (17.2)	4 (5.1)	20 (11.8%)	0.008	
	Total respondents	93	77	170		
Ethnicity	Ashkenazim/Eastern European/White	45 (72.6)	Bedouin Heritage	25 (36.76)	N/A	N/A
	Sephardim/Mizrahim	17 (27.4)	No Bedouin Heritage or unk	43 (63.24)		
	Total Respondents	62		68		

*USD equivalents: 3,000 NIS = 830 USD, 5,000 NIS = 1,400 USD, 10,000 NIS = 2,800 USD, 20,000 NIS = 5,600 USD

Table 2: Relationship between willingness to use recycled water and factors including attitudes towards recycled water, food purchasing habits, and water knowledge among a survey of the general public (n=109) Bethlehem, Palestinian Territories.

		Cooked Produce		**Raw Produce**		**Washing Clothes**		**Bathing**		**Washing dishes**		**Washing produce**		**Cooking**		**Drinking**	
		% Accept	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**
Age	18-20	50.0	0.3	41.2	0.9	33.3	0.5	60.0	0.1	31.8	0.6	68.4	0.01	42.9	0.8	50.0	0.2
	21-30	38.6		32.6		30.9		31.2		33.3		27.1		39.1		23.4	
	31-40	27.3		30.0		18.2		41.7		18.2		27.3		27.3		25.0	
	> 40	12.5		40.0		50.0		33.3		18.2		27.3		33.3		33.3	
I have tested the safety of the water in my home	Agree	50.0	0.1	30.8	0.6	50.0	0.05	46.7	0.9	53.6	0.002	44.8	0.6	65.5	0.02	38.7	0.4
	Did not agree	32.2		37.9		25.8		45.7		16.1		38.2		36.4		28.1	
I have used recycled water that I gathered at my own home	Agree	30.0	0.2	29.6	0.6	41.4	0.4	45.4	0.5	19.3	0.04	42.9	0.6	37.5	0.4	31.2	0.4
	Did not agree	45.9		35.3		30.8		37.2		42.5		36.8		46.3		41.5	
My region of the country has recently experienced water contamination	Agree	34.6	0.6	40.9	0.6	42.9	0.1	35.3	0.7	36.7	0.2	21.9	0.03	48.4	0.1	33.3	0.9
	Did not agree	40.5		34.3		24.4		39.5		23.8		46.1		31.7		34.2	
I have personally experienced the impacts of water contamination	Agree	50.0	0.1	30.0	0.5	15.4	0.03	53.8	0.07	20.7	0.07	38.5	0.9	38.5	0.9	28.0	0.4
	Did not agree	30.9		38.6		40.0		32.7		40.4		37.0		36.7		37.5	
Recycled irrigation water is more likely to pollute downstream waterways	Agree	35.0	0.8	19.0	0.07	26.1	0.2	29.6	0.1	23.1	0.2	30.8	0.4	37.0	0.6	17.4	0.04
	Did not agree	38.8		41.3		40.8		47.2		37.5		40.4		42.9		41.2	
I trust my local utility/wastewater treatment system to test and monitor recycled irrigation water	Agree	62.5	0.01	27.8	0.4	34.5	0.3	53.6	0.04	34.6	0.8	44.4	0.5	36.0	0.5	40.0	0.5
	Did not agree	29.7		39.5		22.6		29.3		32.4		35.9		44.7		31.6	
I trust the private sector to test and monitor recycled irrigation water	Agree	44.4	0.7	52.6	0.03	40.0	0.2	45.4	0.3	31.8	0.9	40.9	0.5	40.0	0.8	28.6	0.7
	Did not agree	38.2		23.7		23.7		32.5		32.5		31.6		37.5		33.3	

Table 3: Relationship between willingness to use recycled water and factors including attitudes towards recycled water, food purchasing habits, and water knowledge among a survey of the general public (n=127) Eilat, Israel.

		Cooked Produce		**Raw Produce**		**Washing Clothes**		**Bathing**		**Washing dishes**		**Washing produce**		**Cooking**		**Drinking**	
		% Accept	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**	**% Accept**	**p-value**
Education	High School	24.1	<0.0001	27.6	0.002	20.6	0.1	14.7	0.3	18.2	0.6	12.1	1.0	11.8	1.0	6.1	0.5
	Some College or Bachelor's	71.9		71.0		31.4		13.9		16.7		13.5		11.1		5.7	
	Graduate Degree	73.3		60.6		44.1		25.7		25.0		11.4		13.9		14.3	
Ethnicity	Ashkenazi/Euro/ White	67.6	0.2	66.7	0.07	41.9	0.02	14.0	0.4	20.9	0.4	14.0	0.3	13.6	0.3	9.5	0.6
	Mizrahim/ Sephardim	46.2		38.5		7.1		6.7		6.7		0.0		0.0		0.0	
I buy food that has been produced in ways that are sustainable for the	Agree	44.8	0.1	46.4	0.4	43.8	0.02	27.3	0.2	19.4	0.9	21.2	0.05	18.8	0.2	13.3	0.2
	Did not agree	61.0		55.7		22.1		16.2		20.3		7.3		8.7		6.0	
The food I want to buy is available and affordable for me	Agree	56.4	0.6	53.8	0.5	34.3	0.5	12.9	0.03	14.3	0.047	14.5	0.4	11.4	1.0	10.0	0.5
	Did not agree	51.4		47.1		27.5		29.3		30.0		7.3		12.2		5.4	
I am familiar with the technologies used to treat and recycle wastewater	Agree	57.9	0.9	57.9	0.5	47.6	0.006	29.3	0.06	34.1	0.009	21.4	0.01	26.2	0.0005	20.0	0.002
	Did not agree	56.6		50.9		22.2		14.0		12.7		4.9		3.2		1.6	
I have used recycled water that I gathered at my own home	Agree	67.7	0.1	73.3	0.007	32.4	0.9	29.4	0.08	42.9	0.003	20.6	0.1	26.5	0.003	16.1	0.1
	Did not agree	50.8		43.9		31.1		14.9		13.2		8.2		5.4		5.5	
I have used recycled water that comes from a wastewater treatment plant	Agree	84.6	0.05	83.3	0.1	80.6	0.001	81.9	0.02	83.1	0.01	79.5	0.1	81	0.004	82.3	0.002
	Did not agree	15.4		35		45.4		18.1		16.9		20.5		19		17.7	
My region of the country has recently experienced lack of water or drought	Agree	60.6	0.4	52.8	0.9	50.0	0.0004	26.3	0.07	28.2	0.046	18.9	0.05	17.9	0.1	10.8	0.5
	Did not agree	51.8		51.8		17.2		12.3		12.5		6.1		6.1		6.2	
My region of the country has recently experienced water contamination	Agree	43.7	0.5	46.7	0.8	18.7	0.6	18.8	0.7	31.3	0.1	25.0	0.047	18.8	0.2	20.0	0.03
	Did not agree	54.0		50.7		24.3		15.1		15.1		6.7		8.1		2.7	
I have personally experienced the impacts of water contamination	Agree	83.3	0.04	75.0	0.1	46.2	0.2	46.2	0.005	30.8	0.3	38.5	0.01	38.5	0.009	38.5	0.001
	Did not agree	51.3		51.2		30.1		14.0		17.4		8.7		8.6		4.4	
Recycled irrigation water is more likely to pollute downstream waterways	Agree	31.3	0.02	35.3	0.06	11.1	0.06	5.9	0.2	5.9	0.1	5.3	0.2	5.6	0.4	5.6	0.7
	Did not agree	64.9		61.4		33.9		21.7		25.0		20.3		16.7		13.8	
Recycled irrigation water is more likely to pollute the soil	Agree	33.3	0.04	20.0	0.003	14.3	0.1	0.0	0.06	14.3	0.7	13.3	1.0	7.1	0.7	7.1	1.0
	Did not agree	62.7		62.3		33.3		23.1		23.4		15.6		13.8		11.1	

Figure 2: Percentage of respondents agreeing with statements regarding food and water perceptions among a convenience sample from Bethlehem, Palestinian Territories (PA) and Eilat, Israel (IL): food and water perceptions, wastewater experience, wastewater perceptions, and willingness to use recycled water. (*=*p*-value< 0.05, **=*p*-value<0.005, ***=*p*-value<0.0005)

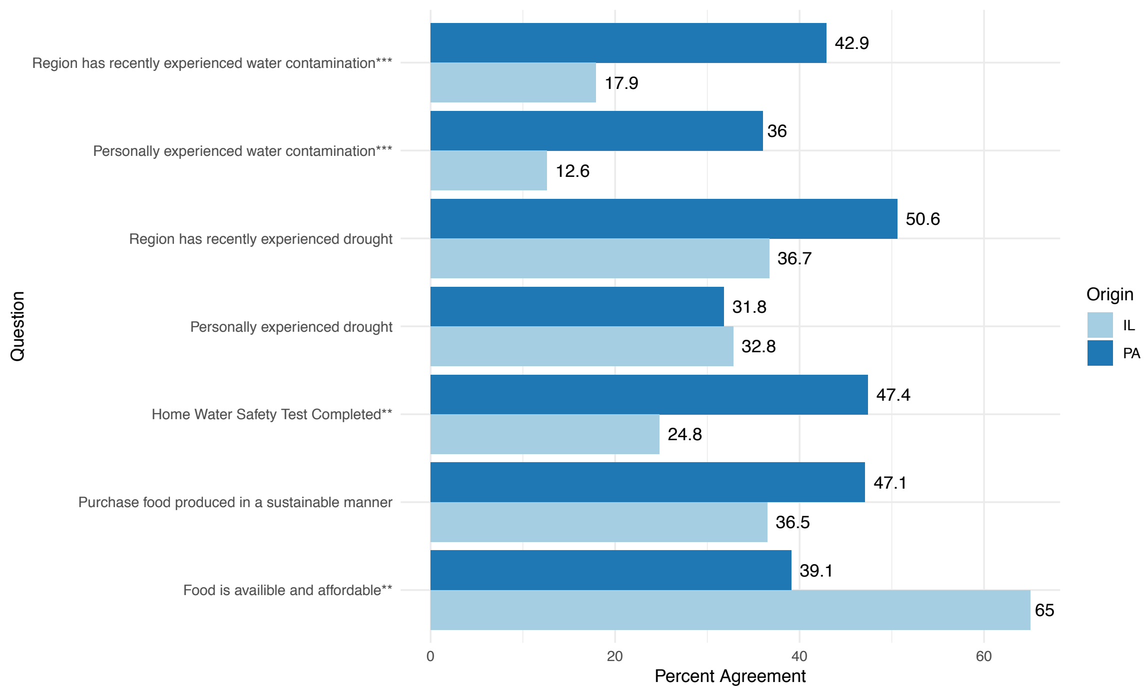


Figure 3: Percentage of respondents agreeing with statements regarding wastewater experience and wastewater perceptions among a convenience sample from Bethlehem, Palestinian Territories (PA) and Eilat, Israel (IL). (*=*p*-value< 0.05, **=*p*-value<0.005, ***=*p*-value<0.0005)

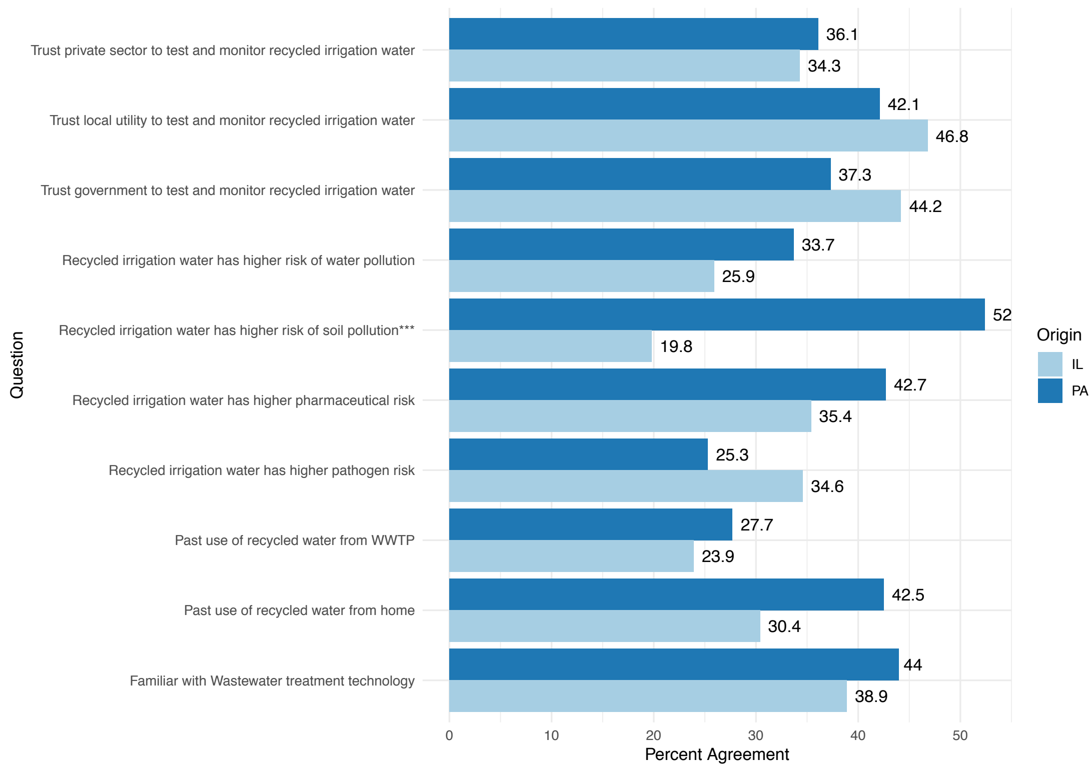
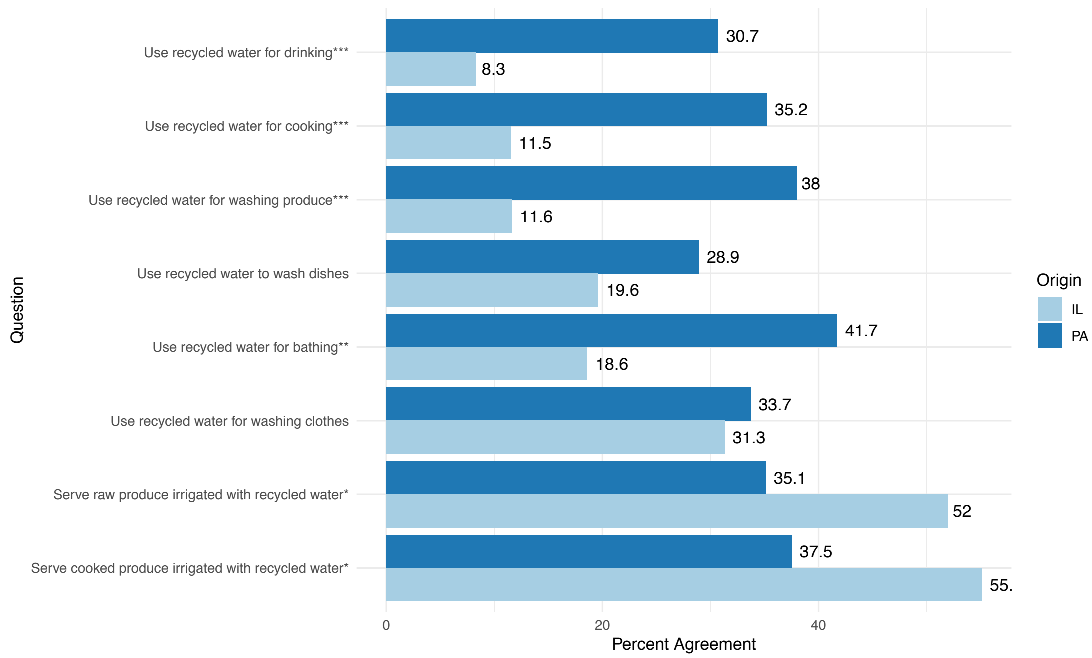


Figure 4: Percentage of respondents agreeing with statements regarding willingness to use recycled water among a convenience sample from Bethlehem, Palestinian Territories (PA) and Eilat, Israel (IL). (*=*p*-value< 0.05, **=*p*-value<0.005, ***=*p*-value<0.0005)



Discussion

Summary of Study Findings

The purpose of this study was to compare Israeli and Palestinian populations with regard to wastewater recycling for household tasks and agricultural irrigation. A greater proportion of Palestinian respondents than Israeli respondents agreed with high-contact options such as cooking and drinking. Overall, most uses garnered less than 50% acceptance; only Israeli respondents specifically were willing to serve raw and cooked produce irrigated with recycled water more than 50% of the time. Higher-contact uses (i.e. cooking, drinking) were more well-accepted among Palestinian respondents than Israeli respondents.

Among the Israeli respondents, experience or familiarity with wastewater recycling and water contamination experience were frequently significantly associated with willingness to use recycled water. Among the Palestinian respondents, personal water contamination experience, home water safety testing, and trust in authorities to monitor recycled wastewater reuse were frequently significantly associated with willingness to use recycled water.

When compared to findings in the literature, our findings among the Israeli respondent population were in line with a previous Israeli survey (Friedler, 2008). Palestinian respondent agreement with serving produce irrigated with recycled water were in agreement with the findings of a 2012 survey in the Bethlehem Governorate, as well as a 2010 survey in the Hebron Governorate (Bethlehem's neighbor to the south) which parsed out agreement with cooked vs. raw produce (Ghanem, 2012; Ghanem et al., 2010). Overall, these findings indicate that both populations are skeptical of wastewater recycling, and education and outreach needs to be different for both populations in order to address relevant concerns and factors which foster acceptance.

Water Contamination Experience

Water contamination experience was highly associated with willingness to use recycled water among Israeli respondents, which is expected given that Hartley (2006) lists “awareness of water supply problems in the community” as one of the key factors contributing to community acceptance of recycled water (Hartley, 2006). It is possible that those who perceive that they have experienced contamination may see recycled water as a “cleaner” source, and thus, may be more willing to use it.

Interestingly, while water contamination experience was also significant within the Palestinian respondent population, the relationship was flipped. The relationship was significant for several potential uses, but those who failed to agree that they had experienced water contamination personally or regionally were more willing to use recycled water. That being said, for other recycling applications agreeing that a home water safety test had been conducted (which may indicate a suspicion of a water contamination issue) was also associated with willingness among Palestinian consumers. Perception of water contamination sources and solutions may warrant further investigation among these two populations.

Future Research

The findings of this survey could be used to inform a shorter, more targeted survey that could be more easily deployed to a larger population of respondents. Efforts should be made to further explore the population's willingness to engage in various forms of water reuse (i.e. assessing the public's willingness to eat livestock that have grazed on wastewater-irrigated fodder) (Al-Sa'ed, Mohammed, & Lechner, 2012; McIlwaine & Redwood, 2011). Furthermore, as stated earlier the relationship between the perception of water contamination and willingness to use recycled water bears further in-depth investigation.

To the author's knowledge, this is the first survey to investigate Israeli ethnicity as it pertains to consumer willingness to use recycled water. Future research could further investigate this relationship by trying to reach an ethnically diverse Israeli population. Research in the West Bank has noted a need for more detailed public education plans regarding wastewater recycling (McNeill, Almasri, & Mizyed, 2009), and a study in Australia also found that factual information campaigns were beneficial (Dolnicar et al., 2010). Thus, future research in these two populations comparing wastewater acceptance before and after factual educational campaigns may be useful. Furthermore, further research could better identify how sub-group characteristics cluster together (i.e. age and environmental attitudes among Israeli respondents) in order to further refine education and outreach.

Limitations

The primary limitation of this research was the convenience sampling methodology. In addition, respondents, especially in the Israeli population, frequently declined to fill in demographic details, which decreased our ability to assess statistical significance. However, as over 100 respondents from each population were sampled using the same survey instrument, this study serves as a foundation for future work which could provide more broadly applicable findings regarding these two populations. Additionally, when designing the survey, most information regarding potential explanatory variables was sourced from surveys outside of the Middle East. Thus, it is possible that, despite closely working with Palestinian and Israeli colleagues, some relevant questions may have been missed (Hartley, 2006; Hummer, 2017; Ross, Fielding, & Louis, 2014).

Conclusions

Our findings suggest that both Israeli and Palestinian populations are willing to use recycled water in various ways, although willingness varies based on the specific intended use as well as between the two respondent populations. Demographic characteristics, attitudes, and previous experiences that are significantly related to willingness to use recycled water differed between our two respondent populations. Based on our findings, we suggest further research to obtain more broadly generalizable results and to continue to confirm and add nuance to understanding of differences in personal characteristics and attitudes that make individuals in the region more or less willing to use recycled water. Given likely increasing water stress in both Israel and the Palestinian Territories, as well as the continued evolution of wastewater treatment technologies, it is important to identify effective and appropriate outreach and communication strategies to increase willingness to use recycled water. Both environmental and development goals can be achieved through innovative water management approaches like treatment and recycling, but these solutions will only have impact if people are willing to use them.

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Chapter 6: Conclusions, Public Health Significance, and Future Work

Conclusions

To the author's knowledge, this dissertation is the first time that a comprehensive assessment has been conducted regarding the presence of antibiotics, herbicides, fecal indicator bacteria, and antibiotic-resistant bacteria in household greywater in the West Bank. Furthermore, this research is novel in that it compares treated greywater directly to the farmers' usual water source, groundwater that has been pumped and stored in an uncovered pond. Additionally, to our knowledge, this is the first study to employ a comparative survey assessing Israeli and Palestinian knowledge and attitudes regarding reused wastewater. The work of this dissertation furthers knowledge regarding the quality of irrigation water produced by small-scale, off-grid systems as well as the public's willingness to consume products irrigated with reused wastewater.

This work demonstrates that antibiotics and herbicides are potentially widespread in household greywater in the West Bank, and even treated greywater can contain higher levels of antibiotics and herbicides compared to conventional irrigation water sources, including surface-stored groundwater. Furthermore, when we investigated bacterial levels and the presence of antibiotic-resistant bacteria in greywater and treated greywater, we noted high levels of bacteria as well as the presence of phenotypic resistance against multiple antibiotics. Resistance to more antibiotics was observed in effluent samples than in pond water samples. These results are critical for assessing the overall quality of this irrigation water source, as well as laying the groundwork for exploring the potential for antibiotic residues present in treated greywater to exert selective pressures on bacterial populations within the water source as well as impacted environmental media, including soil.

Regarding consumer acceptance, Israeli and Palestinian populations agree with various applications of reused water, although agreement levels vary based on use as well as between the two respondent populations. Demographic characteristics, attitudes, and previous experiences that are significantly related to agreement also differ between our two respondent populations. Given likely increasing water stress in both Israel and the Palestinian Territories, as well as the continued evolution of wastewater treatment technology, it is important to identify effective and appropriate outreach and communication strategies to increase acceptance of the use of recycled water. Both environmental and development goals can be achieved through innovative water management approaches like water treatment and recycling, but these solutions will only have impact if people are willing to use them.

The successful completion of these research aims have resulted in an advancement of knowledge regarding the potential health impacts of small-scale, off-grid greywater reuse, as well as societal acceptance of food crops irrigated with reused wastewater in Israel and the Palestinian Territories. No such simultaneous assessment of bacteria, antibiotics, and societal acceptance relating to water reuse has been undertaken in the Middle East. This work advances the general knowledge of the public education needs as well as potential public health impacts of greywater reuse.

Furthermore, these findings are relevant to other countries, including the US. Many parts of the US are coping with or will be coping with water shortages due to mismanagement of water resources and climate change. As a result, the need for reliable access to agricultural irrigation water is likely to continue to be a challenge (Gober & Kirkwood, 2010; Roy et al., 2012; Sapkota, 2019; Vano et al., 2010). As the US is a large, diverse nation, findings from the developed country of Israel can be as applicable as findings from the West Bank, depending on whether the water reuse application in question is needed in an urban or suburban area or in a remote, off-grid community.

Public Health Significance

Food safety

The microbial and chemical quality of reused irrigation water can impact the safety of irrigated food crops and therefore, the health of those eating the irrigated products (Hanning, Nutt, & Ricke, 2009; Liu et al., 2018). Understanding and assessing these issues is critical, as recent foodborne illness outbreaks in the US have been linked to contaminated irrigation water sources (Greene et al., 2008; Hanning et al., 2009; Liu et al., 2018; US FDA, 2018). Treated municipal wastewater is being investigated to characterize its risk to public health via antibiotic resistance and contaminants of emerging concern, but there is a dearth of research in this vein regarding treated greywater, especially in developing settings (Benami, Gillor, &

Gross, 2016; Eriksson, Auffarth, Eilersen, Henze, & Ledin, 2003). During this study, we observed crops being irrigated with the tested treated greywater which could then go on to be eaten raw (i.e. cucumbers, tomatoes). However, if these small-scale systems were not in place it is likely that the risk to food safety would be even higher, as using untreated greywater for irrigation has been observed throughout the West Bank (Bieler, 2016; Ezery, 2016).

It bears noting that while our study design did not include testing of the irrigated produce, recent work from our group showed that when drip irrigation is implemented the transmission of pathogens from irrigation water on to crops is significantly limited (Allard et al., 2019). As these farmers implement drip irrigation, it is therefore necessary to question if these bacteria pose an actual health threat.

Direct Infectious Disease Risk via Wastewater Mismanagement

By diverting, treating, and reusing greywater, pressure is relieved from overburdened, underperforming WWTPs in the West Bank as well as the cesspits which are commonly used throughout the West Bank (McNeill et al., 2009; Mizyed, 2013). Cesspit burden is a concern, as the wastewater from cesspits may go on to be dumped in a nearby wadi, as noted earlier (All-Halih, 2008). Villagers that experienced wastewater dumping in the West Bank have reported that they perceived that they had contracted an infectious disease as a result of the wastewater flooding (Ahmad & Ghanem, 2015).

It has been noted in the Bethlehem region that unmanaged sewage flow is also a substantial risk to springs used for irrigation and drinking water, as well as aquifers as a whole (Reynolds, 2017). Wastewater mismanagement is a substantial contributor to pollution in surface water in the West Bank, and this pollution crosses over into Israel via transboundary streams (Tal et al., 2010). By evaluating the water quality impacts of treating and reutilizing wastewater, the findings of this research can ultimately contribute to alleviating the burden of wastewater mismanagement on the community as well as the overall environment.

Antibiotic Resistance

As noted earlier, previous studies have detected bacteria exhibiting phenotypic resistance to multiple antibiotics in wastewater effluent (Carey et al., 2016; Goldstein et al., 2012; Ottosson, Jarnheimer, Stenström, & Olsen, 2012; Rosenberg Goldstein et al., 2014), and it is known that antibiotic resistance genes can be transferred among environmental bacteria once the bacteria in treated wastewater are introduced into the environment (Martinez, 2009; Rizzo et al., 2013). As WWTP effluent contains both antibiotic-resistant bacteria and detectable levels of antibiotic residues, (Kulkarni et al., 2017, Panthi et al., In Press) it is thus an area of concern that the reuse of treated WWTP effluent for agricultural irrigation may pose a public health threat by introducing additional exposure pathways for antibiotic-resistant bacteria. Exposure to low levels of antibiotics, including the common household antibiotic triclosan, can also drive the selection of antibiotic resistance among bacterial populations (Alistair et al., 2012; Gullberg et al., 2011; Martinez, 2009; Westfall et al., 2019).

Additionally, preliminary occupational studies investigating exposures to antibiotic-resistant bacteria among spray irrigation workers exposed to reused wastewater showed that spray irrigators were more likely to be carriers of coagulase negative *staphylococci* (CoNS), including methicillin-resistant CoNS, than controls (Goldstein et al., 2017). As the prevalence of antibiotic-resistant bacteria detected in effluent in our study was higher than levels in the farmers' standard water source (ponds), more exposure and occupational health studies regarding this issue should be conducted in the West Bank.

Food Security

Abundant irrigation water is needed to fully utilize agricultural lands, and water resources are especially critical in environments where rainfall is not sufficient to meet 100% of water needs. It is estimated that 30-40% of households in the West Bank are considered food insecure, and about a third are reliant on food aid (Batniji et al., 2009; Giacaman et al., 2009; Haddad, 2011). Previous studies have identified large proportions of children reporting inadequate calorie intake, inadequate protein intake, and insufficient micronutrient intake (Abdeen, Greenough, Shahin, & Tayback, 2002; Jildeh et al., 2011). Furthermore, the long-term impacts of malnutrition, namely iron-deficiency anemia, stunting, underweight status, and even wasting, have been observed among West Bank children (Abdeen et al., 2002; Gordon & Halileh, 2013; Jildeh et al., 2011; Tsigga & Grammatikopoulou, 2012).

Water shortages and food insecurity are intricately linked in the West Bank (Haddad, 2011). Studies have found that large proportions of income, even in agrarian households, goes towards food purchases (Halasah, 2017), and, as such, providing more water to grow food at home may alleviate some of this economic burden. While additional irrigation water is not the only answer to food insecurity and malnutrition, off-grid wastewater treatment and reuse systems such as those investigated in this research could be part of a comprehensive public health effort to address overall food insecurity.

Limitations

The primary limitation of the work that addressed Aim 1 and Aim 2 of this dissertation was the small sample size and the fact that, while longitudinal data were collected, it was only collected over nine months. Regarding antibiotic and herbicide residues, the other main limitation was that the grab samples were not true paired samples, as the retention time on the systems varied between 6 and 25 days. True paired samples would allow for more accurate calculations of removal efficiency. Regarding the antibiotic resistance testing, the other main limitation was that only culture-based methodologies were utilized. Thus, bacteria that were viable but non-culturable were missed, as well as bacteria that were not specifically targeted (i.e. Gram-positive bacteria).

The primary limitation of our study that addressed Aim 3 was again the sample size; a larger sample size could have allowed for more power in testing differences based on socioeconomic factors. Additionally, the surveys were only targeted in one community each in the West Bank and Israel and are therefore not representative of all Israeli and West Bank communities. Additionally, our survey was developed using survey instruments that had been developed for use in the

United States and Australia, thus, it is possible that culturally and geographically relevant questions were not asked.

Future Research

While this research introduces novel information into the field of water reuse, it sets the stage for more wide-ranging, broadly applicable research regarding off-grid wastewater reuse as well as consumer acceptance of reuse practices. To address our primary limitation, more systems should be sampled for a longer period of time to establish whether our findings are consistent across the West Bank, if different system types (i.e. constructed wetlands, upflow gravel filtration systems, or other treatment types) perform differently, and to determine if there are seasonal fluctuations. More conventional irrigation water sources should also be sampled, again to provide a broadly applicable comparison to reused greywater. Additionally, off-grid systems are common aid-organization projects; further research needs to be conducted as to how these projects are performing over time and around the world in regards to these and other pollutants. Given the findings of Allard et al., (2019) produce should also be sampled in future research.

Future research regarding consumer acceptance should be conducted to gain greater numbers as well as a wider range of respondents in both Israel and the Palestinian Territories. Efforts should also be made to further explore the population's agreement with various forms of reuse. For example, assessing the public's agreement with eating livestock that have grazed on wastewater-irrigated fodder is an interesting area of future study (Al-Sa'ed, Mohammed, & Lechner, 2012; McIlwaine & Redwood, 2011). Consumer perceptions regarding reused wastewater is especially critical in off-grid communities around the world, where produce will often be consumed close to where it is grown. Therefore, in addition to focusing on urban areas, the perceptions of agrarian, off-grid populations should be taken into consideration.

To the author's knowledge, this is the first survey to investigate Israeli ethnicity as it pertains to consumer trust in reused wastewater. Future research could delve into the impact of ethnicity on wastewater reuse acceptance by trying to reach an ethnically diverse Israeli population. Research in the West Bank has noted a need for more detailed public education plans regarding wastewater recycling (McNeill et al., 2009), and a study in Australia also found that factual information campaigns were beneficial (Dolnicar, Hurlimann, & Nghiem, 2010). Thus, future research in these two populations comparing wastewater acceptance before and after factual educational campaigns may be useful.

Moreover, further research is required regarding how off-grid systems remove contaminants of emerging concern. Biochar and membrane bioreactors have both been indicated as potentially useful for the removal of bacteria and contaminants of emerging concern. Thus, incorporating these approaches into off-grid greywater treatment may be a viable next step (Meuler, Paris, & Hackner, 2008; Moges, Eregno, & Heistad, 2015). Additionally, other research groups have noted that different filtration substrates, flow rate, and plant species can improve the removal of antibiotics (Chen, Wei, et al., 2016; Chen, Ying, et al., 2016). More sampling of various systems should be conducted to assess the real-world efficacy of these different treatment modalities.

It has been shown that even low concentrations of antibiotics in wastewater effluent can select for antibiotic resistance in natural systems (Alistair et al., 2012; Gullberg et al., 2011; Martinez, 2009). Given these findings, additional research is warranted on the impacts of low-levels of antibiotics within off-grid greywater treatment systems on the selection of resistant bacteria in soil and produce in contact with reused greywater. Completing whole-sample DNA extractions and analyzing the DNA with next-generation sequencing (NGS) methodologies would allow for more comprehensive investigation of antibiotic resistance genes. Furthermore, long-term studies coupling NGS with High Performance Liquid Chromatography-Tandem Mass Spectrometry (LC/MS-MS) would allow for the investigation of how patterns of low-levels of antibiotics interface with antibiotic resistance genes.

The behaviors and knowledge of reuse system owners is also a critical area for future research. Prior research in the West Bank also suggested that community capacity and knowledge of the systems is integral to proper functionality of the systems, and research globally has noted that personal behaviors can drive the presence of contaminants of emerging concern in wastewater (Chung & Brooks, 2019; Halasah, 2017). Thus, future work should involve the community to assess their capacity to maintain the systems as well as behaviors that may drive introduction of bacteria and contaminants of emerging concern into household greywater. Individual characteristics of family greywater should be more closely observed to determine whether individual practices contribute to the variation and efficacy of greywater reuse systems. For example, data on household practices regarding the disposal of unused medication could be collected (Chung & Brooks, 2019). If potential causes of variation can be identified, then “best practices” could be developed to guide greywater system owners.

It has been noted that long-term data are needed on these systems. This should also apply to other, less frequently studied, contaminants. Given known environmental contamination issues in the West Bank, future studies should investigate the presence of heavy metals in wastewater samples (Ghanem et al., 2011; Malassa, Al-Rimawi, Al-Khatib, & Al-Qutob, 2014). Additionally, protozoa and helminth infections have been noted in children in the West Bank, thus it is not outside the realm of possibility that these pathogens would be in wastewater from West Bank households (Abu-Alrub, Abusada, Farraj, & Essawi, 2008; Hussein, 2011).

In conclusion, our findings regarding greywater irrigation in the West Bank suggest that current treatment and reuse practices may be insufficient to reduce bacterial loads, remove antibiotic resistant bacteria, and remove contaminants of emerging concern. While the systems in place provide better quality water than simply irrigating with untreated greywater, it is of poorer quality than the pumped groundwater which farmers would use normally. Furthermore, our findings noted a low level of acceptance regarding wastewater reuse in Israel and the West Bank. Overall, these findings as well as future research are important as farmers in the West Bank combat decreasing quality and quantity of groundwater. Farmers both in Israel and the West Bank also rely on a public willing to purchase produce irrigated with treated wastewater. Together, future work must ensure that farmers have access to both safe and abundant irrigation water as well as a public willing to purchase their produce.

Appendices – Supplementary Material

Chapter 3:

Supplemental Table 1: MS/MS parameters, limit of detection, and recovery rate for the 16 analytes.

Compound Name	Precursor Ion	Product Ion	Fragmentor	Collision Energy	Polarity	LOD	Recovery
Alachlor	270.1	238.1	90	10	Positive	0.63	99.2
Atrazine	216.1	174	118	17	Positive	0.3	102.5
Azithromycin	749.5	591.4	188	32	Positive	0.71	84.2
Caffeine	195	138.2	135	30	Positive	0.81	102.3
Ciprofloxacin	332.1	314.1	135	30	Positive	0.88	93.9
Erythromycin	734.5	158.2	90	30	Positive	0.45	82.8
Linezoid	338.2	195	159	28	Positive	0.67	99.8
Oxacillin	402	144	90	30	Positive	1.75	37.1
Oxolinic Acid	262	244	119	20	Positive	1.1	99.2
Penicillin G	335	159.9	100	30	Positive	1.36	68.1
Pipemidic Acid	304	217.4	135	30	Positive	0.76	83.1
Sulfamethoxyzole	254	108	135	30	Positive	1.02	102.7
Triclocarban	313	160	135	10	Negative	1.46	147.8
Tetracycline	445	154.2	190	30	Positive	1.11	87.9
Trifluralin	336.1	236.1	90	30	Positive	0.03	61.7
Vancomycin	725	144	135	10	Positive	0.64	60.3

Chapter 5:

Supplemental Table 1: Counts and percentage of respondents agreeing to statements regarding food purchasing values, water knowledge, wastewater knowledge, drought and water contamination knowledge, wastewater trust, and willingness to use wastewater.

	Israeli		**Palestinian**		
	% Agree	**N**	**% Agree**	**N**	***P*-value**
I buy food that has been produced in ways that are sustainable for the environment	36.5	115	48.2	85	0.1
The food I want to buy is available and affordable for me	65	123	38.9	90	0.0002
I have tested the safety of the water in my home (e.g. lead test)	24.8	109	47.3	74	0.002
I am familiar with the technologies used to treat and recycle wastewater	38.9	108	43.8	73	0.5
I have used recycled water that comes from a wastewater treatment plant	23.9	92	26.6	64	0.70
I have used recycled water that I gathered at my own home (e.g. rain water, gray water)	30.4	112	43.5	85	0.06
My region of the country has recently experienced lack of water or drought	36.7	109	50.6	87	0.05
I have personally experienced the impacts of lack of water or drought (e.g. water use restrictions)	32.8	119	32.5	83	0.9
My region of the country has recently experienced water contamination	17.9	95	43.4	83	0.0002
I have personally experienced the impacts of water contamination (e.g. boil water advisories, warnings to not drink or bathe in tap water)	12.6	111	34.5	87	0.0002
Recycled irrigation water has a higher risk of contamination by pathogens than conventional irrigation water	34.6	81	24.7	77	0.20
Recycled irrigation water has a higher risk of contamination by pharmaceuticals than conventional irrigation water	35.4	79	43.8	73	0.3
Recycled irrigation water is more likely to pollute downstream waterways than conventional irrigation water	25.9	81	33	88	0.30
Recycled irrigation water is more likely to pollute the soil than conventional irrigation water	19.8	81	52.4	82	<0.0001
I trust my local government to test and monitor recycled irrigation water	44.2	104	36.9	65	0.3
I trust my local utility/wastewater treatment system to test and monitor recycled irrigation water	46.8	109	41.3	75	0.5
I trust the private sector to test and monitor recycled irrigation water	34.3	105	34.3	70	1.0
I would serve cooked produce irrigated with recycled irrigation water to my family	55.1	98	36.7	79	0.01
I would serve raw produce irrigated with recycled irrigation water to my family	52	100	34.7	75	0.02
I would use recycled water for washing clothes	31.3	112	32.2	87	0.900
I would use recycled water for bathing	18.6	113	40.4	94	0.0005
I would use recycled water to wash dishes	19.6	112	29.2	89	0.100
I would use recycled water for cooking	11.5	113	36	89	<0.0001
I would use recycled water for washing produce	11.6	112	37.8	90	<0.0001
I would use recycled water for drinking	8.3	109	30.7	88	<0.0001

Supplemental Table 2: All factors tested for association with willingness to use recycled wastewater for irrigating cooked produce, irrigating raw produce, washing clothes, bathing, washing dishes, cooking, washing produce, and drinking, among a convenience sample in Bethlehem, West Bank.

		Cooked Produce	Raw Produce	Washing Clothes	Bathing	Washing dishes	Washing produce	Cooking	Drinking
		% Accept (*p*-value)	% Accept (*p*-value)	% Accept (*p*-value)	% Accept (*p*-value)	% Accept (*p*-value)	% Accept (*p*-value)	% Accept (*p*-value)	% Accept (*p*-value)
Gender	Male	34.5 (0.5)	32.8 (0.5)	28.8 (0.3)	52 (0.2)	32.8 (0.3)	36.4 (0.9)	38.1 (0.9)	33.8 (0.3)
	Female	42.9	41.2	39.3	36.2	21.4	34.8	37.0	21.7
Education	Less than HS	32 (0.7)	31.8 (0.2)	29.6 (0.9)	39.3 (0.8)	37 (0.5)	38.5 (0.7)	30.8 (0.2)	34.8 (0.5)
	High School	34.8	50.0	34.6	44.8	30.4	40.0	52.0	35.7
	Some college or more	41.9	27.3	32.2	37.8	23.1	31.6	33.3	24.3
Age	18-20	50 (0.3)	41.2 (0.9)	33.3 (0.5)	60 (0.1)	31.8 (0.6)	68.4 (0.01)	42.9 (0.8)	50 (0.2)
	21-30	38.6	32.6	30.9	31.2	33.3	27.1	39.1	23.4
	31-40	27.3	30.0	18.2	41.7	18.2	27.3	27.3	25.0
	40+	12.5	40.0	50.0	33.3	18.2	27.3	33.3	33.3
Income	<3,000 NIS	38.5 (0.08)	30.4 (0.9)	31 (1)	58.6 (0.1)	24 (0.4)	48.2 (0.05)	48 (0.2)	38.5 (0.2)
	3,000 - 5,000 NIS	26.3	28.6	30.4	36.0	20.8	18.2	29.2	16.7
	5,001 - 10,000 NIS	66.7	16.7	37.5	20.0	40.0	10.0	20.0	22.2
	> 10,001 NIS	0.0	40.0	25.0	33.3	0.0	25.0	60.0	0.0
Ethnicity	Bedouin Heratige	36.8 (0.8)	36.8 (0.9)	33.3 (0.9)	42.9 (0.5)	29.4 (0.6)	25 (0.1)	52.6 (0.4)	35 (0.7)
	No Bedouin Heritage	33.3	38.5	35.3	52.6	36.1	48.6	41.2	40.6
Religion	Muslim	36.6 (0.9)	33.8 (0.6)	29.1 (0.07)	40.2 (0.1)	29.6 (0.6)	37 (0.5)	39 (0.5)	30.9 (0.8)
	Christian	33.3	50.0	50.0	20.0	16.7	16.7	16.7	33.3
	None	50.0	0.0	100.0	100.0	50.0	50.0	50.0	0.0
I buy food that has been produced in ways that are sustainable for the	Agree	37.5 (0.8)	31 (1)	45.7 (0.1)	48.6 (0.8)	32.3 (0.8)	52.9 (0.09)	52.9 (0.1)	34.3 (0.9)
	Did not agree	40.6	31.0	28.6	45.9	28.9	32.3	33.3	33.3
The food I want to buy is available and affordable for me	Agree	38.5 (0.9)\	32.1 (0.8)	29.6 (0.6)	40 (1)	24.1 (0.2)	25 (0.3)	31 (0.3)	25.8 (0.5)
	Did not agree	39.5	36.1	36.2	39.6	38.3	37.0	43.5	33.3
I have tested the safety of the water in my home	Agree	50 (0.1)	30.8 (0.6)	50 (0.05)	46.7 (0.9)	53.6 (0.002)	44.8 (0.6)	65.5 (0.02)	38.7 (0.4)
	Did not agree	32.2	37.9	25.8	45.7	16.1	38.2	36.4	28.1
I am familiar with the technologies used to treat and recycle wastewater	Agree	37 (0.9)	36 (0.5)	46.4 (0.3)	55.2 (0.2)	34.5 (0.7)	56.7 (0.08)	51.7 (0.3)	37 (0.6)
	Did not agree	35.3	27.3	32.3	38.9	38.2	34.3	39.4	42.9
I have used recycled water that comes from a wastewater treatment plant	Agree	53.8 (0.2)	35.7 (0.7)	42.9 (0.3)	50 (0.9)	35.3 (0.8)	42.9 (0.9)	60 (0.1)	25 (0.3)
	Did not agree	33.3	30.3	27.0	51.2	32.5	40.5	37.5	40.5
I have used recycled water that I gathered at my own home	Agree	30 (0.2)	29.6 (0.6)	41.4 (0.4)	45.4 (0.5)	19.3 (0.04)	42.9 (0.6)	37.5 (0.4)	31.2 (0.4)
	Did not agree	45.9	35.3	30.8	37.2	42.5	36.8	46.3	41.5
My region of the country has recently experienced lack of water or drought	Agree	40 (0.6)	35.1 (0.8)	38.9 (0.3)	36.6 (0.3)	34.2 (0.3)	35.1 (0.4)	41.5 (0.7)	30.8 (0.8)
	Did not agree	34.4	37.9	27.8	47.2	22.9	44.4	37.1	33.3
I have personally experienced the impacts of lack of water or drought	Agree	45.8 (0.4)	38.9 (0.8)	33.3 (0.7)	54.2 (0.2)	30 (0.8)	57.1 (0.06)	39.1 (0.6)	38.1 (0.9)
	Did not agree	34.9	35.7	38.6	38.8	33.3	33.3	45.8	36.2
My region of the country has recently experienced water contamination	Agree	34.6 (0.6)	40.9 (0.6)	42.9 (0.1)	35.3 (0.7)	36.7 (0.2)	21.9 (0.03)	48.4 (0.1)	33.3 (0.9)
	Did not agree	40.5	34.3	24.4	39.5	23.8	46.1	31.7	34.2
I have personally experienced the impacts of water contamination	Agree	50 (0.1)	30 (0.5)	15.4 (0.03)	53.8 (0.07)	20.7 (0.07)	38.5 (0.9)	38.5 (0.9)	28 (0.4)
	Did not agree	30.9	38.6	40.0	32.7	40.4	37.0	36.7	37.5
Recycled irrigation water has a higher risk of contamination by pathogens than conventional irrigation water	Agree	41.2 (0.6)	25 (0.3)	12.5 (0.07)	41.2 (0.9)	16.7 (0.1)	50 (0.2)	29.4 (0.4)	29.4 (0.7)
	Did not agree	33.3	40.0	36.2	42.6	36.2	31.4	40.8	33.3
Recycled irrigation water has a higher risk of contamination by pharmaceuticals than conventional	Agree	28.6 (0.2)	33.3 (0.9)	37 (0.6)	44.4 (0.6)	25.9 (0.3)	51.8 (0.2)	48.1 (0.7)	40.7 (0.4)
	Did not agree	43.7	34.3	43.7	51.3	38.2	35.3	43.2	31.4
Recycled irrigation water is more likely to pollute downstream waterways than conventional irrigation water	Agree	35 (0.8)	19 (0.07)	26.1 (0.2)	29.6 (0.1)	23.1 (0.2)	30.8 (0.4)	37 (0.6)	17.4 (0.04)
	Did not agree	38.8	41.3	40.8	47.2	37.5	40.4	42.9	41.2
Recycled irrigation water is more likely to pollute the soil than conventional irrigation water	Agree	30.6 (0.5)	21.9 (0.1)	32.4 (0.9)	45 (0.7)	40.5 (0.2)	45.7 (0.09)	55.9 (0.06)	43.2 (0.09)
	Did not agree	39.3	40.7	33.3	41.2	27.3	25.8	33.3	23.3
I trust my local government to test and monitor recycled irrigation water	Agree	33.3 (0.7)	35.3 (0.9)	42.9 (0.3)	52.4 (0.3)	25 (0.4)	33.3 (0.1)	52.2 (0.7)	26.1 (0.3)
	Did not agree	38.7	33.3	28.6	37.8	35.3	55.9	46.9	40.0
I trust my local utility/wastewater treatment system to test and monitor recycled irrigation water	Agree	62.5 (0.01)	27.8 (0.4)	34.5 (0.3)	53.6 (0.04)	34.6 (0.8)	44.4 (0.5)	36 (0.5)	40 (0.5)
	Did not agree	29.7	39.5	22.6	29.3	32.4	35.9	44.7	31.6
I trust the private sector to test and monitor recycled irrigation water	Agree	44.4 (0.7)	52.6 (0.03)	40 (0.2)	45.4 (0.3)	31.8 (0.9)	40.9 (0.5)	40 (0.8)	28.6 (0.7)
	Did not agree	38.2	23.7	23.7	32.5	32.5	31.6	37.5	33.3

*USD equivalents: 3,000 NIS = 830 USD; 5,000 NIS = 1,400 USD; 10,000 NIS = 2,800 USD

Supplemental Table 3: All factors tested for association with willingness to use recycled wastewater for irrigating cooked produce, irrigating raw produce, washing clothes, bathing, washing dishes, cooking, washing produce, and drinking, among a convenience sample in Eilat, Israel.

		Cooked Produce	Raw Produce	Washing Clothes	Bathing	Washing dishes	Cooking	Washing produce	Drinking
		% Accept (p-value)	% Accept (p-value)	% Accept (p-value)	% Accept (p-value)	% Accept (p-value)	% Accept (p-value)	% Accept (p-value)	% Accept (p-value)
Gender	Male	48.7 (0.1)	47.4 (0.3)	22.0 (0.07)	12.2 (0.3)	19.5 (0.9)	7.1 (0.5)	11.6 (0.7)	9.8 (0.7)
	Female	65.4	57.4	39.3	20.6	19.0	12.5	9.8	6.7
Education	High School	24.1 (<0.0001)	27.6 (0.002)	20.6 (0.1)	14.7 (0.3)	18.2 (0.6)	11.8 (1)	12.1 (1)	6.1 (0.5)
	Some College or Bachelor's Degree	71.9	71.0	31.4	13.9	16.7	11.1	13.5	5.7
	Graduate Degree	73.3	60.6	44.1	25.7	25.0	13.9	11.4	14.3
Age	18-20	33.3 (0.2)	44.4 (0.1)	38.5 (0.7)	25 (0.5)	36.4 (0.8)	18.2 (0.5)	36.4 (0.1)	10 (0.4)
	21-30	51.6	50.0	25.7	25.0	20.0	16.7	8.3	5.7
	31-40	70.6	58.8	25.0	10.0	15.0	5.0	10.0	5.3
	41-50	60.0	50.0	33.3	0.0	16.7	16.7	16.7	16.7
	51-60	73.3	80.0	26.7	12.5	12.5	12.5	13.3	18.8
	60+	42.9	34.8	43.5	21.7	20.8	4.2	4.2	4.3
Income	<5,000 NIS	50 (0.2)	50 (0.5)	42.9 (0.9)	14.3 (0.9)	28.6 (0.2)	14.3 (0.9)	0 (0.7)	0 (0.9)
	5,001 - 10,000 NIS	43.8	41.9	31.3	21.2	27.3	8.8	14.7	9.7
	10,001 - 20,000 NIS	72.0	65.4	28.1	15.2	9.1	12.1	6.1	6.3
	>20,000 NIS	53.8	57.1	26.7	13.3	13.3	6.7	6.7	6.7
Ethnicity	Ashkenazi/Euro/White	67.6 (0.2)	66.7 (0.07)	41.9 (0.02)	14 (0.4)	20.9 (0.4)	13.6 (0.3)	14 (0.3)	9.5 (0.6)
	Mizrahim/Sephardim	46.2	38.5	7.1	6.7	6.7	0.0	0.0	0.0
Religion	Jewish	52.6 (0.4)	48.1 (0.06)	26.7 (0.05)	15.9 (0.2)	18 (0.07)	9 (0.05)	11.1 (0.2)	6.9 (0.1)
	Christian	33.3	33.3	57.1	28.6	14.3	14.3	0.0	0.0
	None	72.7	90.0	54.5	36.4	50.0	36.4	30.0	30.0
	Other	66.7	66.7	0.0	0.0	0.0	0.0	0.0	0.0
I buy food that has been produced in ways that are sustainable for the environment	Agree	44.8 (0.1)	46.4 (0.4)	43.8 (0.02)	27.3 (0.2)	19.3 (0.9)	18.8 (0.2)	21.2 (0.05)	13.3 (0.2)
	Did not agree	61.0	55.7	22.1	16.2	20.3	8.7	7.3	6.0
The food I want to buy is available and affordable for me	Agree	56.4 (0.6)	53.8 (0.5)	34.3 (0.5)	12.9 (0.03)	14.3 (0.04	11.4 (1)	14.5 (0.4)	10 (0.5)
	Did not agree	51.4	47.1	27.5	29.3	30.0	12.2	7.3	5.4
I have tested the safety of the water in my home	Agree	57.1 (0.9)	55 (0.9)	30.8 (0.9)	25.9 (0.2)	23.1 (0.6)	19.0 (0.5)	7.4 (1)	8.3 (0.7)
	Did not agree	55.9	53.5	29.5	15.4	17.9	9.0	10.5	6.5
I am familiar with the technologies used to treat and recycle wastewater	Agree	57.9 (0.9)	57.9 (0.5)	47.6 (0.006	29.3 (0.06)	34.1 (0.00	26.2 (0.0005	21.4 (0.01)	20 (0.002)
	Did not agree	56.6	50.9	22.2	14.0	12.7	3.2	4.9	1.6
I have used recycled water that comes from a wastewater treatment plant	Agree	84.6 (0.05)	83.3 (0.1)	80.6 (0.001	81.9 (0.02)	83.1 (0.01	81 (0.004)	79.5 (0.1)	82.3 (0.002)
	Did not agree	15.4	35	45.4	18.1	16.9	19	20.5	17.7
I have used recycled water that I gathered at my own home	Agree	67.7 (0.1)	73.3 (0.007)	32.3 (0.9)	29.4 (0.08)	42.9 (0.00	26.5 (0.003)	20.6 (0.1)	16.1 (0.1)
	Did not agree	50.8	43.9	31.1	14.9	13.2	5.4	8.2	5.5
My region of the country has recently experienced lack of water or drought	Agree	60.6 (0.4)	52.8 (0.9)	50 (0.0004)	26.3 (0.07)	28.2 (0.04	17.9 (0.1)	18.9 (0.05)	10.8 (0.5)
	Did not agree	51.8	51.8	17.2	12.3	12.5	6.1	6.1	6.2
I have personally experienced the impacts of lack of water or drought	Agree	60.6 (0.5)	57.6 (0.5)	39.5 (0.2)	21.1 (0.6)	26.3 (0.2)	15.8 (0.3)	10.8 (1)	8.3 (1)
	Did not agree	53.1	50.0	27.0	17.3	16.2	9.3	12.0	8.2
My region of the country has recently experienced water contamination	Agree	43.7 (0.5)	46.7 (0.8)	18.7 (0.6)	18.8 (0.7)	31.3 (0.1)	18.8 (0.2)	25 (0.047)	20 (0.03)
	Did not agree	54.0	50.7	24.3	15.1	15.1	8.1	6.7	2.7
I have personally experienced the impacts of water contamination	Agree	83.3 (0.04)	75 (0.1)	46.2 (0.2)	46.2 (0.005)	30.8 (0.3)	38.5 (0.009)	38.5 (0.01)	38.5 (0.001)
	Did not agree	51.3	51.2	30.1	14.0	17.4	8.6	8.7	4.4
Recycled irrigation water has a higher risk of contamination by pathogens than conventional irrigation water	Agree	47.6 (0.1)	41.7 (0.1)	16 (0.1)	8.3 (0.1)	20 (0.9)	12 (1)	11.5 (0.7)	8 (0.7)
	Did not agree	67.3	61.2	32.0	23.5	21.6	13.7	15.7	12.2
Recycled irrigation water has a higher risk of contamination by pharmaceuticals than conventional irrigation water	Agree	60.9 (0.9)	40 (0.1)	24 (0.5)	8.3 (0.1)	24 (0.5)	8 (0.7)	11.5 (0.7)	4.2 (0.4)
	Did not agree	59.6	60.4	32.0	21.6	17.6	13.7	16.0	12.2
Recycled irrigation water is more likely to pollute downstream waterways than conventional irrigation water	Agree	31.3 (0.02)	35.3 (0.06)	11.1 (0.06)	5.9 (0.2)	5.9 (0.1)	5.6 (0.4)	5.3 (0.2)	5.6 (0.7)
	Did not agree	64.9	61.4	33.9	21.7	25.0	16.7	20.3	13.8
Recycled irrigation water is more likely to pollute the soil than conventional irrigation water	Agree	33.3 (0.04)	20 (0.003)	14.3 (0.1)	0 (0.06)	14.3 (0.7)	7.1 (0.7)	13.3 (1)	7.1 (1)
	Did not agree	62.7	62.3	33.3	23.1	23.4	13.8	15.6	11.1
I trust my local government to test and monitor recycled irrigation water	Agree	59 (0.5)	52.5 (0.9)	30.2 (0.9)	20.5 (0.7)	20.5 (0.7)	11.4 (0.7)	9.5 (0.5)	7.1 (0.7)
	Did not agree	52.8	51.8	30.9	17.9	17.9	14.3	15.8	10.9
I trust my local utility/wastewater treatment system to test and monitor recycled irrigation water	Agree	56.8 (0.8)	53.2 (0.9)	31.2 (0.7)	16.7 (0.5)	20.8 (0.9)	10.2 (0.5)	8.3 (0.4)	8.3 (1)
	Did not agree	54.7	51.9	34.5	21.4	21.4	14.3	16.1	9.4
I trust the private sector to test and monitor recycled irrigation water	Agree	60.6 (0.5)	57.6 (0.5)	35.3 (0.6)	20.6 (0.6)	23.5 (0.6)	17.6 (0.2)	11.8 (1)	6.1 (0.7)
	Did not agree	53.3	50.8	30.8	16.7	19.7	9.0	10.6	9.4

*USD equivalents: 3,000 NIS = 830 USD; 5,000 NIS = 1,400 USD; 10,000 NIS = 2,800 USD; 20,000 NIS = 5,600 USD

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