OCCURRENCE OF *SALMONELLA* IN CANALS DELIVERING IRRIGATION WATERS USED FOR PRODUCE PRODUCTION

By

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Contents

List of Tables

List of Figures

Abstract

With the increase in produce consumption in recent years, the risk of foodborne illness increases as well. It is estimated that 1,940 reported cases of foodborne illnesses were associated with the consumption of produce in the United States in 2015. *Salmonella enterica* is one of the top bacterial foodborne pathogens of concern and causes gastroenteritis estimated to have caused about 1,300 reported produce-borne outbreaks in the US/year. One of the ways *Salmonella* can travel to produce fields and contaminate produce is via irrigation water. The objective of this study was to detect the presence of *Salmonella* and indicator organisms of fecal contamination including *Escherichia coli*, coliforms, and coliphages in constructed canal systems which deliver irrigation waters for produce production. A total of 355 irrigation water samples were collected from various irrigation canals in Southern Arizona from January 2017 to August 2017. All samples were tested for general water quality or physiochemical parameter and analyzed using enrichment media, selective media, and confirmational methods for the presence of *Salmonella enterica*. This analysis yielded 11.6% (41/355) positive isolates with an average concentration of 4.10 MPN/100 ml. *E. coli* and coliforms were detected via IDEXX Colilert ®kit and IDEXX Quanti-Tray/2000® resulting in 97% (344/355) and 100% (355/355) positive samples, respectively. F+ and somatic coliphages were detected using the FastPhage™ MPN Quanti-tray method and resulted in 35.4% (62/175) and 60.5% (127/210) positive samples, respectively. The results of this study indicate that *Salmonella* and fecal indicator organisms were found at detectable levels in irrigation waters used in crop production. This implies the potential to cause foodborne illnesses, if the crops are contaminated with irrigation water that contains pathogens. A quantitative microbial risk assessment analysis was performed, using a Monte Carlo simulation and the exponential dose response model, to determine the spread of *Salmonella* to lettuce from irrigation water. The probability of causing infection annually was estimated as 2.1×10^{-7} and 5.1×10^{-5} for concentrations of 4.1MPN/100 ml and 1000MPN/100 ml at day 0 following the irrigation event, respectively. This is lower than the EPA standard of 1 in 10,000 per year, making this concentration of *Salmonella* in irrigation canal waters relatively safe for produce consumers. However, additional sampling and data would help further determine potential risks of irrigation water and contamination potentials.

Literature Review

Salmonella enterica

Salmonella enterica is a Gram negative, facultatively anaerobic bacterium belonging to the family *Enterobacteriaceae*. *S. enterica* is commonly found as a commensal bacterium in the digestive tracts of most vertebrates (Ibarra & Steele-Mortimer, 2009a). *Salmonella* is the number one cause of bacterial foodborne illnesses in the world and number one in the most reported deaths and hospitalizations yearly in the United States due to bacterial foodborne illness (CDC, 2015a). *Salmonella* can be found in many types of contaminated foods including meat products, produce, and ready to eat foods (Foodsafety.gov, 2016). *Salmonella* has even been detected in surface waters used to irrigate crops (Levantesi et al., 2012). Salmonellosis is the illness caused by *Salmonella* and can be mild to severe depending on the individual and the concentration of the bacterium present (AFIA, 2010). Signs and symptoms of salmonellosis include gastrointestinal problems such as vomiting, diarrhea, abdominal cramps, nausea, and fever. These symptoms can last for several days to a week, individuals can continue to shed the bacterium via feces after symptoms subside for up to two weeks (CDC, 2016b; Giannella, 1996; Giese, 2013). This bacterium is also very hardy and can survive in many environments and conditions, making *Salmonella* a successful pathogen of concern (Pasquaroli et al., 2013; Patrone et al., 2013; Ramamurthy et al., 2014).

Salmonella Foodborne Outbreaks

Salmonella is ranked number two within the top five foodborne pathogens in the United States (CDC, 2018a). According to the CDC, *Salmonella* is thought to be the cause of about 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths reported annually in the United States (CDC, 2018l). It is estimated that one million of these illnesses may be linked to food (CDC, 2018l). Currently in 2018, there have already been 12 reported *Salmonella* outbreaks. Three of these were from meat-based products, two from live animals, five from produce, and two from grains/cereals. One outbreak involving raw turkey products is ongoing but so far has 90 reported illnesses and 40 hospitalizations across 26 states caused by multidrug resistant (MDR)

Salmonella Reading. The source of the outbreak has not yet been identified; however, the strain of MDR *Salmonella* Reading has been traced back to live turkeys, raw turkey products, and raw turkey pet food products (CDC, 2018i). In North Carolina, *Salmonella* Braenderup caused an outbreak with 45 reported illnesses and 11 hospitalizations across 10 different states linked to chicken eggs. Over 200,000,000 possibly contaminated eggs were recalled by a company in North Carolina, which is a huge economic loss as well as a major public health problem (CDC, 2018d). A more serious poultry product related outbreak originating from a meat packing company in Iowa resulted in 265 reported illnesses, 94 hospitalizations, and one death. The product was a ready to eat deli chicken salad with vegetables product that became contaminated with *Salmonella* Typhimurium. This contaminated product was found in eight states (CDC, 2018h). Another *Salmonella* outbreak that occurred in 2018 was from Kellogg's honey smacks cereal. The cereal contaminated with *Salmonella* Mbandaka caused 100 reported illnesses and 30 hospitalizations across 33 states. The investigation is still ongoing, and the cereal has been recalled (CDC, 2018f).

Produce Related *Salmonella* outbreaks

Although *Salmonella* is mainly of animal/mammal origin, fruits and vegetables (produce) can still become contaminated. An earlier study showed that *Salmonella* caused over 2,200 reported illnesses in the United States over a six-year period (2009-2015) from contaminated seeded vegetables (Dewey-Mattia et al., 2018; Scallan et al., 2011a). *Salmonella* has been shown to contaminate produce via feces from animals/mammals, irrigating with contaminated water, improper food handling/processing, and other routes (Foodsafety.gov, 2016; Hanning et al., 2009; Lapidot & Yaron, 2009; Luo et al., 2015). In 2015, the CDC reported a large *S.* Poona outbreak which was linked to imported cucumbers from Mexico. This outbreak caused a total of 907 cases, 204 hospitalizations, and six deaths in 40 states, making it one of the largest in recent years in the United States (CDC, 2016a; Siegner, 2015). In 2017, there was another produce outbreak in the U.S. from contaminated Maradol papaya imported from Mexico. Five serotypes of *Salmonella* (Thompson, Kiambu, Agona, Gaminara, and Senftenberg) caused 220 illnesses, 68 hospitalizations, and one death across 23 states (CDC, 2017). In 2018 there was a

Salmonella outbreak caused by pre-cut melons sold in grocery stores traced back to Indiana. This outbreak of *Salmonella* Adelaide caused 77 reported illnesses, 36 hospitalizations, and no deaths (CDC, 2018c). Two separate cases of *Salmonella* foodborne illness from imported coconut products also occurred this year. One outbreak was caused by *Salmonella* Typhimurium and resulted in 14 reported illnesses and three hospitalizations across six states from contaminated dry coconut (CDC, 2018g). The other outbreak was from frozen shredded coconut contaminated with *Salmonella* Newport which resulted in 27 illnesses and six hospitalizations across nine states (CDC, 2018e). A current outbreak involving produce in a pre-made pasta salad containing pasta, carrots, celery, cucumbers, green pepper, onion, and mayonnaise sold in Hy-Vee grocery stores was caused by *Salmonella enterica*. This contaminated pasta mostly made of vegetables is the cause of 79 reported illnesses and 18 hospitalizations across nine states (central northern U.S.). The outbreak is still under investigation (CDC, 2018j). One of the main reasons behind produce outbreaks is that most of the time, produce is consumed raw and even under some processing, pathogens such as *Salmonella* are still able to survive (Finn et al., 2013; Waldner et al., 2012).

Survival of *Salmonella enterica* in the Environment

There are many *Salmonella* foodborne illnesses due to this bacterium's ability to survive in many conditions including foods and various environments. *Salmonella* uses several survival tactics to thrive including biofilm formation, a viable but non-culturable (VBNC) state, and pili/fimbriae. Biofilm formation is a defense mechanism used by bacteria and other microbes that group together on a surface and produce an extracellular polymeric substance that forms a protective barrier (Donlan, 2002). Biofilm formation can protect microbes from various chemicals, antimicrobials/antibiotics, antiseptics, and sanitizers (Joseph et al., 2001; Gilbert, 2002; Scher et al., 2005). VBNC is a state of slowed metabolism in stressed bacteria. It allows bacteria such as *Salmonella* to survive in harsh, unfavorable conditions (Oliver, 2009; Pinto et al., 2015). Once conditions such as moisture, available nutrients, general living conditions (pH, temperature, etc.) return to normal, the bacterium will then revert out of VBNC and metabolic functions will return to normal (Gupte et al., 2003; Reissbrodt et al., 2002;

Reissbrodt et al., 2000). Fimbriae and pili are filamentous structures that bacteria use to help form biofilms, they help with cell to cell aggregation and adhesive colonies (Collinson et al., 1993; Collinson et al., 1991). These resilient structures are resistant to detergents, proteolytic enzymes, bases, and boiling; they are thought to aid in the survival of *Salmonella enterica* through the harsh conditions of the mammalian digestive tract (Collinson et al., 1993; Collinson et al., 1991).

A study done by You et al. (2006) showed that *Salmonella* could survive in manure-amended non-sterilized soil and manure-amended sterilized soil for up to 400 days in laboratory-created field conditions (You et al., 2006). Islam et al. (2004) showed that *Salmonella* Typhimurium was able to survive in several types of soils and on the surfaces of lettuce and parsley for about 230 days (Islam et al., 2004). The persistence of *Salmonella* in field environments suggests the possibility of transportation and contamination of *Salmonella* to other fields and surface waters (Islam et al., 2004). Another study showed that *Salmonella* could survive in a laboratory-created river water (microcosm) for 31 days. This persistence allows the bacteria time to be ingested by a host or for deposition onto a favorable surface (Santo Domingo et al., 2000). In addition to survival in waters, another study showed that *Salmonella* is still able to express virulence genes while in various surface waters (Nutt et al., 2003). Polo et al. (1998) investigated the prevalence of *Salmonella* as a fecal indicator in environmental water samples. They collected various freshwater and saltwater samples and found that freshwater rivers yielded the highest *Salmonella* positives. *Salmonella* is very hardy in the environment, but it is also able to survive on many surfaces for longer periods of time. One study showed that dehydrated *Salmonella* can survive on a plastic surface for 100 weeks under refrigeration. The study concluded that this is possible because the bacterium reverted to the VBNC state (Gruzdev et al., 2012). Another study that tested the prevalence of *Salmonella* in fish feed factories over three years. During this period, there were two isolates of *Salmonella* serovars Montevideo and Agona that were found every year. They were confirmed via PFGE and repeatedly isolated, indicating that they survived for three years in the Norwegian fish farm factories (Nesse et al., 2003). *Salmonella* has also been tested on paper products. One study demonstrated that *Salmonella* could survive on paper discs for 35 and 70 days at 25°C

and 30°C respectively (Hiramatsu et al., 2005). The same study also showed that *Salmonella* could survive on these paper discs for 24 months of storage at 4°C (Hiramatsu et al. 2005). *Salmonella* has also been found to survive on stainless steel surfaces for 4 days (Kusumaningrum et al., 2003). A *Salmonella* contaminated wet sponge to stainless steel transfer was performed, followed by placing cucumber and chicken on the surface of the stainless steel. The transfer rates to the food items were consistently 20% to 100%, respectively (Kusumaningrum et al., 2003). This study demonstrated a factory/warehouse scenario where contamination can occur very easily as well as the survival and transfer of pathogens such as *Salmonella* on surfaces or equipment (Kusumaningrum et al., 2003).

The idea is that *Salmonella* is introduced to food products via raw ingredients and is able to survive in low numbers into the finished cooked or processed product and is then able to cause foodborne illness (Finn et al., 2013). *Salmonella* can survive in ready to eat dry products with a moisture content as low as 8%, an example being chocolate in which certain types of *Salmonella* have been able to survive for up to 15 months (Tamminga, et al., 1977; Werber et al., 2005). Another study showed that *Salmonella* can also survive in low moisture vacuum sealed foods as well. *Salmonella* was able to survive for eight months on a product called halva (A sesame seed dessert product) which was vacuum sealed and has a moisture content of 0.18 aw (available water) (Kotzekidou, 1998). Peanut butter is another food product that *Salmonella* can survive in and has been responsible for many foodborne illnesses. One study demonstrated that *Salmonella* can survive in peanut butter for up to 24 weeks at refrigeration or room temperature. Also, of the seven commercial varieties tested, the natural peanut butter showed the least *Salmonella* survival rates (Burnett et al., 2000). Mentioned above are just several examples of how *Salmonella* can survive in/on harsh environments, surfaces, and even low moisture food products for long periods of time.

Previous studies on *Salmonella* in irrigation water and outbreaks

Salmonella outbreaks on produce have increased over the years due to the increase in human population and the demand for fresh produce (Harris et al., 2003). There are many ways *Salmonella* can be transferred to produce in fields, but one of the easiest routes is via irrigation

water (Steele & Odumeru, 2004). A study done in New York tested various types of surface waters for the prevalence of *Salmonella.* Out of 18 sites for a total of 123 samples collected, there was a 43% *Salmonella* positive recovery (Jones et al., 2014). These samples were collected over a two year period (2010 and 2011) during the growing season in New York which is May through October (Jones et al., 2014). Another study done in Georgia by Haley et al. (2009) looked at the prevalence of *Salmonella* along two large rivers monthly over a years' time. Out of a total of 72 surface water samples collected, 57 (79.2%) were positive for *Salmonella* (Haley et al., 2009). Thirteen different serotypes of *Salmonella* were identified from all samples and there was also a positive correlation with rain and *Salmonella* densities. Many of the *Salmonella* positives were thought to be the result of agricultural, livestock and reptile sources (Haley et al., 2009). Another study in Florida and Georgia looked at the prevalence of *Salmonella* monthly from 10 irrigation ponds (Luo et al., 2015). The researchers recovered 28.2% *Salmonella* positive isolates out of 635 samples collected, as well as a positive correlation of *Salmonella* positives with rain and warmer temperatures (Luo et al., 2015). A 2.5-year study in the central California coast region was done where only 6% (6/96) *Salmonella* positive isolates were recovered from various irrigation water sources (Benjamin et al., 2013). One study was able to trace back two *Salmonella* Newport outbreaks from contaminated tomatoes in 2002 and 2005; the source was from irrigation ponds in Virginia (Greene et al., 2008). These two outbreaks combined caused almost 600 illnesses. The specific strain of *Salmonella* was identified via pulsed-field gel electrophoresis (PFGE) and the irrigation water and contaminated tomato isolates matched (Greene et al., 2008). Also, as mentioned previously, *Salmonella* Typhimurium with curli can successfully be transferred from irrigation water to produce (Lapidot and Yaron 2009). The study showed the transfer of *Salmonella* to parsley leaves via contaminated irrigation water and its ability to survive for 21 days post-inoculation (Lapidot & Yaron, 2009). This type of situation occurring naturally could easily cause produce foodborne outbreaks which is a major public health concern to those who consume raw produce.

Escherichia coli

Escherichia coli is a Gram negative rod shaped facultative anaerobic bacterium belonging to the family *Enterobacteriaceae* (Gerba, 2009; Jang et al., 2017). *E. coli* is commonly found in the healthy digestive tracts of many mammals and humans as commensal bacterium. *E. coli* is also known to be a pathogen of major concern. There are seven serotypes of *E. coli* known to cause illness in humans; however, the majority of *E. coli* are non-pathogenic (CDC, 2018k; Cooper et al., 2014). *E. coli* is also found in the environment such as in water, manure, and soil (Jang et al., 2017; van Elsas et al., 2011). *E. coli* is environmentally stable and has been found to survive for 27 days on a leafy green surface and 80 days in soil manure and even is able to grow/reproduce in the environment (Erickson et al., 2010; Franz et al., 2005; van Elsas et al., 2011). Like *Salmonella, E. coli* can survive in various environments because they are able to go into a "dormant" VBNC state (Ding et al., 2017; van Elsas et al., 2011). *E. coil* can survive in low pH, high or low temperatures, limited resources/nutrients, and low moisture content (Bergholz & Whittam, 2007; Franz et al., 2005; Semenov et al., 2007; van Elsas et al., 2011). Studies show that *Salmonella* is able to out-survive *E. coli* in various environmental conditions, although they are both durable microbes (Franz et al., 2005; Semenov et al., 2007).

E. coli can enter the environment via animal and human feces and wastewater effluents and is often used as an indicator organism of fecal contamination in various water sources (CDC, 2018k; Jang et al., 2017). Although generic or non-pathogenic *E. coli* does not cause illness, it can accompany other organisms that do (Rock & Rivera, 2014). Most produce foodborne pathogens come from the intestines and feces of animals and humans, similar to generic *E. coli* which makes it a good fecal indicator organism (Uyttendaele et al., 2015). Fecal contamination in irrigation water can come from wastewater, animal trespassing, runoff agricultural water, and additional sources (Rock & Rivera, 2014; Savichtcheva & Okabe, 2006; Uyttendaele et al., 2015). Water is such a valuable resource and our supply is limited. The earth is made up of 70% water; however, only 2.5% is freshwater and we only have access to about 1% (National Geographic, 2018; USGS, 2016). Of all freshwater on earth, 70% is used for agricultural purposes (USGS, 2018). It is important to maintain good water quality, mainly because it is such a valuable limited resource.

E. coli is a good indicator of fecal contamination in waters and is easier to detect than other enteric pathogens. The U.S. Environmental Protection Agency (EPA) and the FDA's Food Safety Modernization Act (FSMA) have set guidelines/regulations for *E. coli* concentrations in surface waters to reduce the risks of humans being exposed to pathogens (EPA, 2003; FDA, 2018). In 2003, the EPA set guidelines for *Escherichia coli* in surface freshwaters where the population of *E. coli* cannot exceed 126 *E. coli* cells per 100 ml of water from a geometric average of five samples collected (EPA, 2003). Recently FSMA and the FDA also proposed statistical threshold levels of *E. coli* to be less than 410 CFU per 100 ml of water for a single sample (FDA, 2018). If levels are above 126 *E. coli*/100 ml, then it is recommended that the water source is not used until the water is treated and levels are below the limit (FDA, 2018).

E. coli found in irrigation waters

A study done by Luo et al. (2015), sampled 10 irrigation ponds in Florida and Georgia and found an overall geometric mean of generic *E. coli* of 6.26 MPN/100 ml of water. From two of the irrigation ponds, there were a total of six samples that exceeded the 410 MPN/100 ml of *E. coli* single sample threshold out of 635 samples over a two-year period (Luo et al., 2015). There was a weak correlation between *E. coli* and *Salmonella* (r=0.34) positives. A study mentioned previously by Benjamin et al. (2013) also detected generic *E. coli* from various irrigation water sources in central California. A 78% (199/255) positive recovery of *E. coli* was detected and the arithmetic mean was 710 CFU/100 ml of water. This is above the EPA guidelines (Benjamin et al., 2013). The study found that generic *E. coli* was not correlated with the presence of *Salmonella* and in this case, it would not be a good indicator organism of *Salmonella* (Benjamin et al., 2013). Another study found *E. coli* in 16 out of 18 irrigation ponds sampled in New York, a 33% (40/123) positive recovery of *E. coli* was found. This study determined that there was not a strong correlation between *E. coli* and *Salmonella* positives from the same samples (Jones et al., 2014).

Coliforms

Coliforms are a group of non-pathogenic bacteria usually found in the digestive tract and feces of humans and animals. Coliforms are found in water, plant material, and soils (CDC, 2013). Coliforms are in the family *Enterobacteriaceae* and the three commonly seen organisms besides *E. coli* are *Enterobacter, Citrobacter,* and *Klebsiella* (Gerba, 2009; Uyttendaele et al., 2015). Total coliforms are used to detect contamination from failed control measures (Uyttendaele et al., 2015). If coliforms are present in water samples, this is generally indicative of recent human/animals fecal contamination or soil contamination (CDC, 2013; Rock & Rivera, 2014). Similar to *E. coli,* the presence of coliforms can indicate the possible presence of pathogenic microbes in the same contaminated water source. The EPA standard for fecal coliforms in drinking water is 0/100 ml but for recreational freshwater the standard is 126 *E.coli*/ 100 ml water for five samples over a 30 day period (US EPA, 2012).

Fecal Coliforms found in irrigation water

The study mentioned above by Luo et al. (2015) also looked at the presence of coliforms in the 10 irrigation ponds in Florida and Georgia and found an overall geometric mean of 10.3 CFU/100 ml of water over a one-year period. There was also a correlation between fecal coliforms and *E. coli* (r=0.69) and the fecal coliforms also correlated with *Salmonella* similar to *E. coli* (Luo et al., 2015). Another study conducted in Brazil examined the prevalence of fecal coliforms on lettuce from irrigation water. The study collected 129 samples of lettuce and of these, 17% showed high concentrations of fecal coliforms (Takayanagui et al., 2000). Another study done in Mexico looked at ready-to-eat (RTE) salads irrigated with untreated sewage water and found a 99% (129/130) fecal coliform recovery. The average fecal coliform concentration was 571 fecal coliform/gram (Castro-Rosas et al., 2012). These samples were purchased from various restaurants in Mexico in popular tourist areas which included chain, local, and small restaurants (Castro-Rosas et al., 2012). One study in Canada in 2002 examined 27 irrigation water sources located on 17 farms in Ontario and found mean values of 8,559 coliforms/100 ml of water from 494 samples collected and 162 fecal coliforms/ 100 ml of water from 446 samples collected (Steele, Mahdi, & Odumeru, 2005). This study collected samples

over a one-year period. The study reported that the irrigation water samples were acceptable for fecal coliforms between 70 to 96% of samples collected according to Canadian standards (Steele et al., 2005).

Coliphages

Coliphages are a type of bacteriophage or a bacterial virus. They have also been used as indicator organisms of fecal contamination as well as virus indicators (Gerba, 2009; Savichtcheva & Okabe, 2006). They are similar in size, structure, movement in the environment such as aquatic environments, and behavior to enteric viruses (Savichtcheva and Okabe 2006; Funderburg and Sorber 1985; Gerba 2009; US EPA 2015). Coliphages can be an indicator of the presence of enteric pathogenic viruses, since they all come from the intestines and feces of warm blooded animals and humans (US EPA, 2015). Coliphages are non-pathogenic, they are host-specific, and they generally infect *E. coli.* Coliphages are a preferred indicator organism because they are easier and less expensive to detect, culture, and quantify compared to viral enteric pathogens (Gerba, 2009; US EPA, 2015). Coliphages can be detected rapidly using many different methods including single and double agar layer methods (EPA Method 1601 and 1602), polymerase chain reaction (PCR) methods, culture latex agglutination and typing (CLAT), and FastPhage® modified 1601 method (US EPA, 2015). There are two coliphages of interest which are F-specific and somatic coliphages. These are considered more reliable indicators of fecal contamination and represent human enteric pathogenic viruses best compared to other viral indicators (Havelaar et al., 1990; US EPA, 2015). These coliphages are resistant to environmental factors as well as water disinfection such as chlorination and wastewater treatment processes to the same extent as pathogenic enteric viruses (Feng et al., 2003; Gerba, 2009; US EPA, 2015).

Coliphages found in irrigation water

Currently there are no regulations for limits on concentration of viruses in surface waters. However, there is a regulation by the ADEQ of one virus particle/40 liters of water in Arizona for reclaimed water used for the irrigation of food crops (ADEQ, 2016; Gerba, 2009; Gerba & Choi, 2006). Espinosa et al. (2009) conducted a study in Mexico City and found a strong correlation of the recovery of coliphages and enteroviruses (P-value=0.0182) in the same samples. The study also showed that bacterial fecal indicators showed no relationship in detecting viral presence in irrigation water. There was also no significant relationship between coliphages and rotavirus (P-value = 0.1502) (Espinosa et al., 2009). These samples were collected from various sources of irrigation water and well water from November-February and May-October over a two-year period (Espinosa et al., 2009). Another study done in California collected water samples for a year from 15 different locations along Newport bay which included water samples from watershed sources in urban and agricultural areas (Jiang et al., 2007). Jiang et al. (2007) detected an average of F+ coliphage MPN of 1.79/100 ml. The coliphages were detected in higher numbers during the winter season after storms (t test, $P<0.01$) compared to summer. In this study, there was a correlation between the presence of fecal indicator bacteria and coliphages; however, there was no statistical relationship between fecal indicator bacteria (FIB) and coliphages and human viruses (Jiang et al., 2007). Another study in South Carolina looked at the presence of coliphages over a years' time from various watershed samples from 96 surface water stations (Stewart-Pullaro et al., 2006) analyzed a total of 117 surface water samples were analyzed and an average of 52 plaque forming units (PFU)/100 ml for F-specific coliphages and 313 PFU/100 ml for somatic coliphages was found. These coliphages were typed for microbial source tracking and the results suggested that the contamination of coliphages in the surface water was from human fecal pollution (Stewart-Pullaro et al., 2006). In Mexico, a study was done where 146 irrigation water samples were analyzed for coliphage in a large irrigation canal. Coliphages were detected in less than 30% of samples with an average of 141 PFU/100 ml (Gortáres-Moroyoqui et al., 2011). In another study, coliphages were used as a model to estimate concentrations of hepatitis A virus (HAV) from irrigation water to produce to determine microbial risk assessment (Stine et al., 2005). A worst-case scenario using coliphages was shown if the produce was picked and consumed the day after irrigation; the concentration

of Hepatitis A virus (HAV) would be about $2.5x10^{-5}$ MPN/100 ml which would result in an annual risk of 1 in 10,000. The risk of 1:10,000 is acceptable, however, the concentrations were conservative, and conditions may be different under industry practices. Stine et al. (2005) determined that furrow irrigation could lead to a higher risk of crop contamination compared to subsurface drip irrigation (Stine et al., 2005). Coliphages are a good indicator of fecal contamination in water but there is still some uncertainty if they are also good indicators of enteric viruses.

Irrigation Canals

Arizona provides about $23.3 billion revenue in agriculture, and $3.2 billion in the US is from produce(AZDA, 2018). During the winter, Southern Arizona produces about 90% of the leafy greens consumed in the U.S., which requires a lot of land and water (Murphree 2013; Yuma Visitors Bureau and the AFB 2016). In Southern Arizona, there are more than 230,000 acres of agricultural land which produces more than 175 different crops and seeds (Yuma Visitors Bureau and the AFB 2016). The main source of water used to irrigate these crops comes from the Colorado river (YID, 2018). The Colorado river supplies seven western U.S. states and northern Mexico. The river basin is 246,000 square miles long (CRWUA 2018a). Arizona receives about 2.8 million acre-feet (maf) of water from the Colorado river and Southern and Central Arizona receives and uses about 1.5 maf (CRWUA, 2018a; USBR, 2015). Southern Arizona has about 271 miles of irrigation canals. One of the largest irrigation canals from the Colorado river connected by the Imperial dam is the All-American Canal, which is 80 miles long (Imperial Irrigation District, 2017; NASA.GOV, 2017; USBR, 2018). The All-American Canal is cement-lined for 23 miles and it ranges from 150-200 ft wide, 7-20 ft deep, and has a 175 ft drop (Imperial Irrigation District, 2017). In Southern Arizona, there are several types of irrigation canals which include main canals (canal connected/branch off the main source), lateral canals (connected to the main canal), sub lateral canal (connected to the lateral canals), and drainage canals (collect the runoff water from irrigated fields). There are various sizes of canals that hold several inches

deep of water to 20 feet deep. Irrigation canals can be lined (usually cement lined) or unlined (dirt canal) (FAO, 2000).

Water quality standards for surface waters in Arizona

The EPA sets standards for water quality in effect of the Clean Water Act in surface waters. The EPA works with the Arizona Department of Environmental Quality (AZDEQ), Water Quality Division to set surface water quality standards in Arizona (Department of Environmental Quality, 2017). The water quality standards for *E. coli* are no more than a geometric mean of 126 CFU/100 ml (at least five samples collected within 30 days) or a single sample with a max of 235 CFU/100 ml. The standards for pH in agricultural irrigation for crops is between 4.5 to 9.0 (Department of Environmental Quality, 2017). The salinity/total dissolved solids standards for the Colorado river at the Imperial Dam (Southern Arizona region) has a limit of 879 mg/L. These are the current standards for surface waters in Arizona as of 2016 (Department of Environmental Quality, 2017).

Occurrence of *Salmonella* in Canals Delivering Irrigation Waters used for Produce Production

Introduction

It is estimated that 632 pounds of fruits and vegetables are consumed yearly per capita in the United States alone (Produce for Better Health Foundation (PBH), 2015; Statista, 2018). Along with the high consumption of produce comes a risk of foodborne outbreaks. The Centers for Disease Control (CDC) reported 1,940 cases of foodborne illnesses associated with the consumption of produce in the United States in 2015 (CDC, 2015). *Salmonella* causes severe gastroenteritis and was estimated to cause about 1,300 cases of these produce-borne illnesses in 2015 (CDC, 2015). There are various ways that enteric bacterial pathogens can contaminate produce in fields including fecal contamination from animal sources, manure, and contaminated irrigation water (Beuchat, 2006; Steele & Odumeru, 2004; Strawn et al., 2013).

Currently there are few studies on the detection and quantification of *Salmonella* in irrigation waters (Pachepsky et al., 2011). A study done by Duffy et al.(2005) in Texas confirmed *Salmonella* in 16/170 (9.4%) of the irrigation water samples. Studies done in Greece and Alberta, Canada detected *Salmonella* in 6% of irrigation waters (Arvanitidou et al., 1997; Johnson et al., 2003). Greene et al. (2008) traced the contamination source of tomatoes involved in an outbreak in Virginia to pond water used to irrigate the tomatoes. This tomato outbreak impacted 26 states and caused a total of 510 illnesses (Greene et al., 2008). Behravesh et al. (2011) identified the source of an outbreak of *Salmonella* in the United States linked to peppers as contaminated irrigation/agriculture water in Mexico, which caused 1,500 cases of salmonellosis in 2008 (Behravesh et al., 2011). These studies demonstrated that *Salmonella* could be present in irrigation water worldwide and can cause foodborne outbreaks.

Arizona is the third largest produce producer in the nation and supplies 90% of the leafy greens to the United States during the winter season (Yuma Visitors Bureau and the Arizona

Farm Bureau, 2016). Since Southern Arizona is such a large supplier of produce, it is important to understand the prevalence of *Salmonella* in waters used for crop irrigation. This study was designed to determine the occurrence of *Salmonella* in constructed canal systems which deliver irrigation waters for produce production and to quantify the potential risks from contamination using a risk assessment model. In addition, the prevalence of *Escherichia coli*, coliforms, and coliphages as indicator organisms of human fecal contamination was also assessed.

Materials and Methods

Study area

Southern Arizona receives most irrigation water from the Colorado river. In addition, Southern Arizona receives the last of this water before it leaves the United States and enters Mexico. Samples of irrigation water were collected from 53 sites along the canals in Southern Arizona during the winter and summer from January to August 2017. These sites were chosen based on various factors such as a mixture of cement-lined canals, unlined (earthen embankments) canals and historical data regarding the occurrence of generic *E. coli* in these waters (Gerba & Choi, 2006; Bright et al., unpublished data 2014, 2015, 2017). The types of canals included main canals, lateral canals, sub-lateral canals (a branch off a lateral canal), and drainage canals (collecting return flows from produce fields). Canals were also selected based on a variety of locations including rural locations such as in fields far from the urban areas and urban and suburban locations such as in neighborhoods or near stores/buildings. Canal sizes varied depending on canal type and ranged from 0.07 to 1.6 meters in width depending on the type of canal. The depth of water ranged from 0.25 to 1.6 meters deep in the main canals to 0.07 to 1.3 meters in the lateral, sub lateral, and drainage canals. There were 12 sites that were completely lined or lined only near the collection site and 12 that were unlined that were in rural areas. These rural areas were surrounded by produce fields, dirt, and roads (Figure 2, Image D). There were also suburban sites which included 11 canals that were lined or lined at the collection site and six unlined canals. Suburban area canals included features such as a few houses or stores nearby, minimal auto/human traffic, paved and dirt roads, and some produce fields (See Figure

1, Images A, B, & D). The following canal sites were in urban locations, which include seven canals that were lined or lined at the collection site and five unlined canals. Urban canal sites were areas near high traffic, stores, houses, sidewalks, and paved roads (See Figure 3, Images A & C). Of the total sampling locations, seven were drainage canals; five of these were unlined and two were lined, and three were in suburban locations and four were in rural areas.

Eighteen of the sampling sites with cement-lined canals (locations M4, 1, 2A, 2B, 3A, 3C, 6A, 10A, 10B, 14A, 15A, 15B, 16A, 18A, 22A, 22B, 23A, 24B) and eighteen of the sampling sites with unlined canals (4A, 4B, 5A, 5B, 7A, 7B, 8A, 8B, 9, 11A, 11B, 13, 17, 20A, 20B, 21, 23A, 23B) were observed to be frequented by various animals such as ducks, other birds, and fish (See Figure 2, Images A & B). There were 19 cement-lined canals (M1, M2, M4, M5, 1, 2A, 2B, 3A, 3C, 6A, 10A, 10B, 12, 12B, 14B, 15A, 16A, 22A, 24B) and 22 unlined canal sites (4A, 4B, 5A, 5B, 7A, 7B, 8A, 8B, 9, 11A, 11B, 13, 16B, 17, 18B, 18C, 19, 20A, 20B, 21, 23A, 23B) that contained algae or plant debris (See Figure 1, Image B). Since all canals are open/exposed and the area of the canals are exposed to wind and weather, there was trash observed in each canal location at least once. Items found in the canals included: plastic bottles, Styrofoam, shoes, shopping carts, and other types of garbage that did not belong (See Figure 1, Image E).

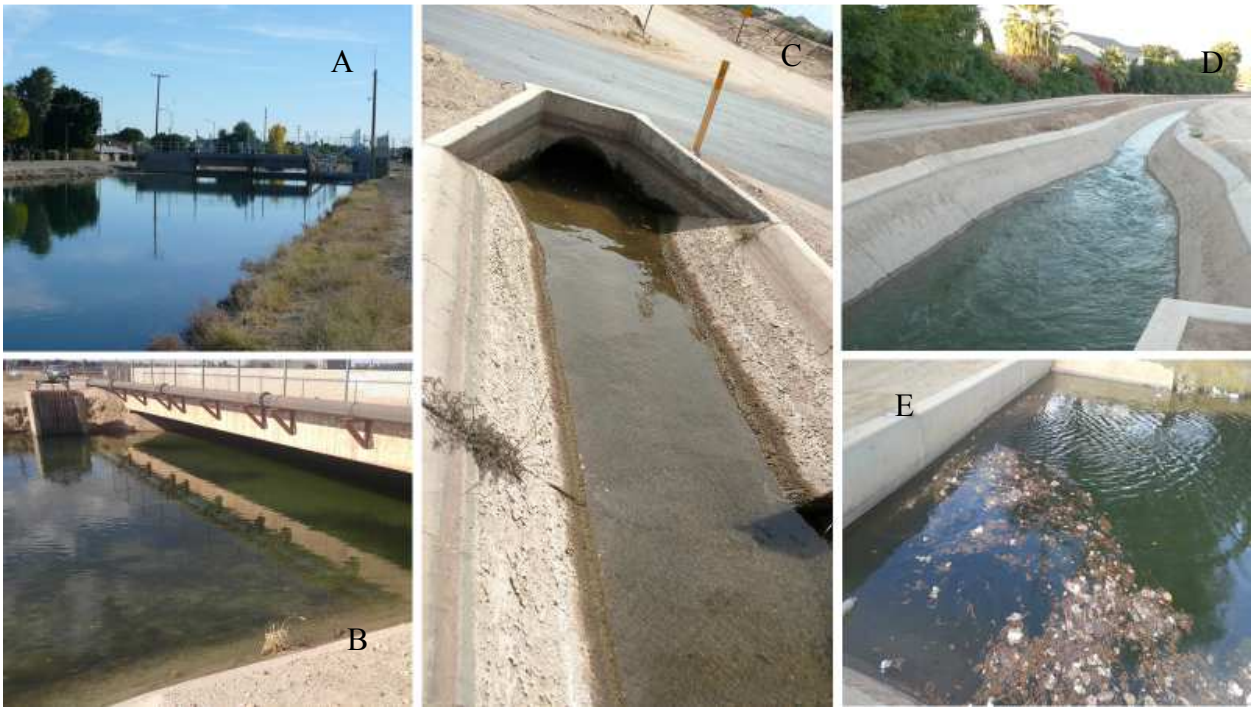
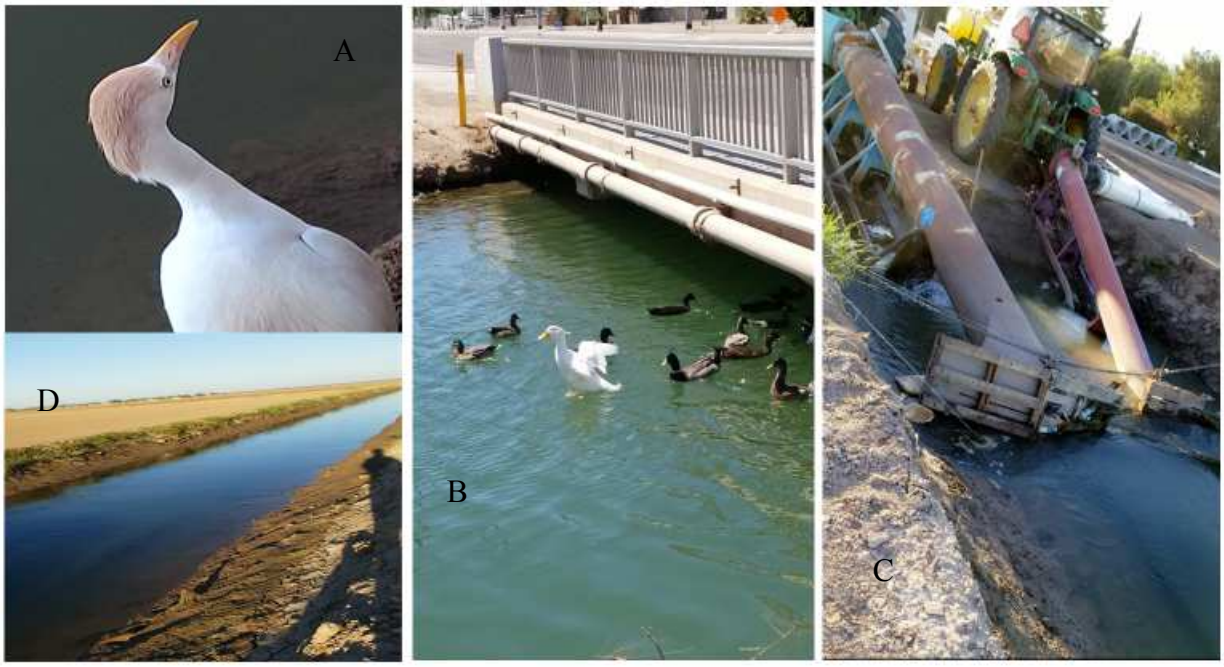


Figure 1: Images of irrigation canals: Images A and B are unlined slower flowing irrigation canals with bridges and traffic over them in a suburban area. Image A shows a canal that is very wide and has some houses to the left and lower banks; image B shows a canal that has plant debris/algae at the bottom and is in a suburban



area. Images C and D are cement lined irrigation canals with steeper canal banks; image C shows that the canal has low water levels and slow flowing and is very close to a paved road; image D is a faster flowing canal with higher water levels, the water is a nice blueish color and it looks clean. It is a suburban canal because it is behind a neighborhood but there is a wall with vegetation separating them both. Image E shows a canal that has a lot of plastic trash such as bottles, containers and Styrofoam cups. It also has a lot of plant debris such as sticks and leaves and a type of bio-like film. The water level in image E was full and green/blue in color the canal area was lined at the collection site.

Figure 2: Images of irrigation canals: Images A and B show types of birds that live in and near the canals. Picture A shows a cattle egret on the bank of an unlined canal in a rural area. Picture B shows an assortment of ducks in an unlined urban irrigation canal. Picture C shows some construction being done on an unlined irrigation canal. Image D Shows an irrigation canal that is unlined and in a rural area.

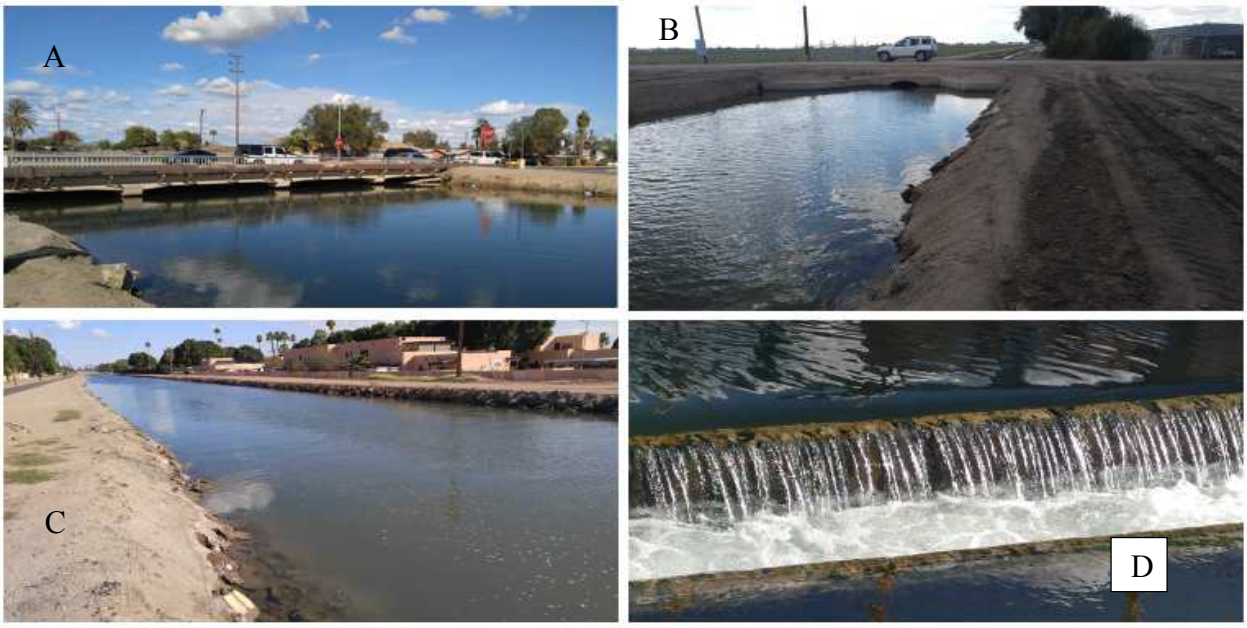


Figure 3: Images of Irrigation canals: Image A shows an irrigation canal in an urban area with a bridge and traffic. It is very wide and is unlined and is surrounded by neighborhoods and has ducks that reside under the bridge. There is also trash in the canal. Image B shows a suburban unlined irrigation canal; the canal is large and is near some houses and warehouses on the right side and fields on the left side. Image C shows a wide unlined urban irrigation canal near apartments, houses, businesses, and a bike path. The water in image C is a brownish/turbid color; the water flow is slow near the canal banks. Image D shows a mixing station in an Irrigation canal, the water here is moving fast.

Sample collection

Two one-liter sterile bottles were used to collect irrigation water from each site. Data was also recorded for the time of sampling such as general canal characteristics (cement lined or unlined canals, urban, suburban, or rural areas) and the current conditions (a visual observation of a slow, medium, or fast flow rate of water, high or low water levels, visual observations of the water quality, color of the water and cloudiness in the canals, animals or people near/in canals, littered area, etc.). A total of 355 irrigation water samples were collected and processed over a one-year period.

Physical/chemical and Microbial determinations

A Multi-Parameter Tester (PCSTestr Eutech model- PCSTEST35-01X441506) and a VWR® Traceable® Hygrometer/ Thermometer (VWR® Radnor, PA USA) were used at the time of collection for each sample and included determination of the pH, conductivity, total dissolved solids, salinity, air relative humidity, and water and air temperature. Samples for microbiological assays were kept on ice until processed (within 24 hours of collection). *E. coli* and coliform levels were also determined using an IDEXX Colilert ®kit and IDEXX Quanti-Tray/2000® (IDEXX Laboratories, Inc. Westbrook, Maine USA). The presence of coliphages were determined by using the FastPhage™ MPN Quanti-tray method (Charm Sciences, Lawrence, MA). Coliphages are a type of bacteriophage or a bacterial virus found in feces, their host is *E. coli*. They have also been used as indicator organisms of fecal contamination as well as virus indicators (Gerba, 2009; Savichtcheva & Okabe, 2006). Turbidity was determined using a Hach Turbidimeter (Model 2100AN Turbidimeter. Hach Company. Loveland, CO, USA). The presence and levels of *Salmonella* were determined using a most probable number (MPN) method using four different volumes. A one-liter volume was first passed through a membrane filter (0.45 µm pore size) (Millipore, Merck KGaA, Darmstadt, Germany). Three replicates of 100 ml volumes of irrigation water were also passed through separate membrane filters (0.45 µm pore size) (Millipore, Merck KGaA, Darmstadt, Germany). The filters were then placed into separate tubes containing 10 ml of tryptic soy broth (TSB, Hardy Diagnostics. Santa Maria, CA, USA). Ten and one ml samples were added directly to 10 ml of TSB without filtering. All volumes were tested in triplicate except for the 1-liter volumes and incubated @37°C for 18-24 hours. After incubation, the samples in TSB were vortexed and then 1 ml of each was transferred into separate tetrathionate broth tubes (TTB, EMD Millipore Corporation. Billerica, MA USA) containing an Iodine/potassium and Iodine solution (potassium iodine Sigma-Aldrich™ St Louis, MO; Iodine crystalline 99.5% solid Alfa Aesar™ Haverhill, MA) and incubated at 37˚C for 18 to 24 hours. Samples were then vortexed and transferred from Tetrathionate broth (TTB) to Rappaport Vasilliadis broth (RVB, Becton, Dickinson and Company™ BD™ Pont de Claix, France) and incubated at 41.5˚C for 48 hours. The samples in Rappaport Vasilliadis broth were then transferred to Xylose Lysine Desoxycholate agar (XLD Granucult, Merek™, Germany) by the streaking for isolation method. The XLD plates were incubated at 37° C for 24 to 48 hours and

were then evaluated for the presence of *Salmonella*. Presumptive *Salmonella* isolates (red colonies with a black center) were frozen in a glycerol (Sigma-Aldrich™. St Louis, MO) and Brain Heart Infusion (BHI, Oxoid, UK) solution to preserve them and stored at -80°C. The presumptive isolates were subsequently thawed on ice and streaked on *Salmonella* Chromagar plates (CHROMagar™ Paris, France) to eliminate *Proteus* contamination followed by testing with API 20E biochemical strips (bioMérieux®, Durham, NC) for additional confirmation. Final confirmation was done using PCR (Rahn et al. 1992 and Alam et al. 2009) with *invA* primers F 3'-GTGAAATTATCGCCACGTTCGGGCAA- 5' and R 3'TCATCGCACCGTCAAAGGAACC- 5' (Invitrogen Carlsbad, CA USA) for the detection of the *Salmonella* invasion (*invA)* gene. A positive band for the *invA* gene shows up at 262 KB on the Agarose LE, Analytica gel (Promega Corporation Madison, WI).

Quantitative Risk Assessment

A risk assessment analysis was done in the current study to determine the risk of infection from *Salmonella enterica* due to the consumption of crops or produce water irrigated with potentially contaminated water in Southern Arizona. The dose-response model chosen was the exponential model [P(infection)=1-exp(-k*N)], which was found on the Quantitative Microbial Risk Assessment (QMRA) wiki website (Rose et al., 2017). The *Salmonella enterica* serotype Newport was chosen because it is has been linked to produce- or water-based cases (Tamrakar, 2013). A dose was calculated using an equation (Dose= ((Concentration of *Salmonella* in water*)* x (max microbe transfer to lettuce) *x* (1-Die off rate)) $^{\text{days on lettuce}}$ x (Intake of lettuce in g/day)) and a Monte Carlo simulation in excel using a software called Simulacion 4.0© (Varela 2003). Two point estimates were provided from Stine et al. (2005) - the maximum transfer of bacteria from irrigation water to lettuce with a value of 0.00011% as well as the bacterial die off rate/day which was 0.35 log/day. The stochastic variables that were selected were the ingestion rate of lettuce in grams/day and the ingestion rate of leafy vegetables in g/day (See Table 10) (US EPA, 2018). The concentrations of *Salmonella* used in this risk assessment were the average concentration as well as the highest concentration recovered

from the current study. The dose was calculated for the concentration of *Salmonella* on lettuce and leafy vegetables combined during day one and day seven and then put into the exponential equation above. The day seven time point was selected because this is the minimal time it takes for produce to be harvested and to reach the consumer.

Statistical analysis of results

Data were analyzed using Microsoft® Excel 2016. To make comparisons on the results of the data, the ANOVA test was used to determine whether a difference was observed in microbial and general water quality parameters in *Salmonella* positive samples and *Salmonella* negative samples. Two-tailed Student's t-tests were also used to determine if there were significant statistical differences when comparing various parameters in different types of canal characteristics such as cement-lined versus unlined canals. Pearson's linear correlation coefficient tests were performed on all the microbial and physiochemical parameter results to determine if there were any parameters that associated with one another that could influence *Salmonella* positive results. Values <1 were changed to 0.9 for coliphages for correlation. All microbial numbers were log transformed to ensure normal distribution. For the microbial risk assessment, the software Stimulacion 4.0© in Excel was used to run a Monte Carlo simulation for the dose of consuming *Salmonella* on lettuce to calculate the risk of infection using the exponential dose model (Tamrakar, 2013; Varela, 2003).

Results

Overall findings

The recovery of *Salmonella enterica* positive isolates from irrigation canals in Arizona was 11.6% (41/355) (See Table 1). Additionally, 97% (344/355) of all samples were positive for *Escherichia coli* and 100% (355/355) of samples were positive for the presence of total coliforms. Coliphages were detected as well in the irrigation water; 35.4% (62/175) of samples were positive for F+ coliphages and 60.5% (127/210) were positive for somatic coliphages (See

Table 1). *E. coli* was found at overall average concentrations of 64 MPN/100 ml (arithmetic mean) and 27 MPN/100 ml (geometric mean) in irrigation water samples and total coliforms were found at average concentrations of 1,649 MPN/100 ml (arithmetic mean) and 1,441 MPN/100 ml (geometric mean). Somatic phages and F+ phages were detected in lower concentrations in many of the irrigation canals with overall average concentrations of 3 MPN/100 ml (arithmetic mean) and 2 MPN/100 ml (geometric mean) and 59 MPN/100 ml (arithmetic mean) and 12 MPN/100 ml (geometric mean), respectively (See Table 1). And the overall concentrations of *Salmonella enterica* in all sampled irrigation canals were 4 MPN/100 ml (arithmetic mean) and 0.02 MPN/100 ml (geometric mean). The highest *Salmonella* concentration found was 1,000 MPN/100 ml in one sample and the range for positive *Salmonella* samples was from 0.01 MPN/100 ml to 1,000 MPN/100 ml. The *Salmonella* MPN was semi-quantitative because of the process of enrichment, detection, isolation, and confirmation where positive isolates may have been lost or overlooked.

Main, Lateral/Sublateral, and Drainage canals

There were three types of canals sampled in this study which included the collection of- 157 samples from main irrigation canals, 148 samples from lateral/sublateral canals, and 50 samples from drainage canals. The average water quality overall was higher in main and sublateral canals than in drainage / return flow canals (See Tables 3, 5, and 6). A significant difference (*P* value ≤ 0.05) in all three canal types was found in pH for general water parameters. There was a significant difference between main and lateral/sublateral canals in air temperature (*P* value=0.02). There was a significant difference between lateral/sublateral and drainage canals in water temperature (*P* value=0.006). There was a significant difference between main and lateral/sublateral canals compared to drainage canals in the following parameters - conductivity (main and drainage *P* value = 0.007; lateral/sublateral and drainage *P* value = 0.008), salinity (*P* value = 0.007 and *P* value = 0.01, respectively), and total dissolved solids (*P* value = 0.007 and *P* value = 0.009, respectively) in which the values were higher in

drainage canals. There was no significant difference for turbidity or relative humidity between all three canal types (See Tables 3 and 5).

Coliforms were significantly more abundant (P-value ≤ 0.05) in drainage or return flow canals (2,340 MPN/100 ml arithmetic, 2,330 MPN/ 100 ml geometric) compared to both main and lateral/sublateral irrigation canals (1,497 MPN/100 ml and 1,569 MPN/100 ml arithmetic, 1,492 MPN/ 100 ml and 1,264 geometric, respectively) (See Tables 3, 4, and 6). The geometric mean for *Escherichia coli* was detected in significantly higher concentrations in the main canals (43 MPN/100 ml) compared to lateral/sublateral canals (20 MPN/100 ml) and no difference was observed for the drainage canals (33 MPN/100 ml) when compared to either. For the somatic coliphages, the concentrations were significant (P-value ≤ 0.05) between the main canals (5 MPN/100 ml) compared to both lateral/sublateral (2 MPN/100 ml) and drainage canals (2 MPN/100 ml) for both the arithmetic and geometric means. Looking at the geometric mean of F+ coliphages, there was only a significant difference between lateral/sublateral (20 MPN/100 ml) and drainage canals (5.6 MPN/100 ml). For *E. coli,* F+ coliphages, and *Salmonella* concentrations, there were no significant differences found between all three types of canals when looking at the arithmetic mean. For *Salmonella,* there were also no differences in the geometric means of theses canal types (See Table 6). *Salmonella* was found most often in drainage canals 14% (7/50), followed by lateral/sublateral 13.5% (20/148), and finally main canals 9% (14/157) (See Table 3 and 6).

Lined and Unlined Canals

Of the 53 irrigation canal sites sampled, 29 canals were cement-lined and 24 were unlined; 157 samples were collected from lined canals and 198 samples were collected from unlined canals (See Table 3). Overall, there were two significant differences (P-value ≤ 0.05) in general water quality parameters in lined irrigation canals compared to unlined canals for pH and turbidity (See Table 7). In the unlined canals, the turbidity (7.1 NTU) was statistically different compared to the lined canals (4.6 NTU). The pH was statistically different and more alkaline in the lined canals (8.3) on average compared to the unlined canals (8.1) (See Table 7). For the microbial

water content, there were no significant differences found between *E. coli*, coliforms, somatic coliphages, or F+ coliphages in the comparison of lined and unlined canals. *Salmonella* was found slightly more often in lined canals 12.7% (20/157) compared to 10.6% (21/198) in unlined canals. The differences could have been slightly higher in lined canals compared to unlined because *Salmonella* could potentially have more areas to attach in the unlined canals where they can hide in the soils or sediment, instead of free floating in the lined canals. The microbial content was slightly higher in unlined canals, but these differences were not significant (See Tables 4 and 8). Overall there were no significant findings between lined and unlined canals that would really set them apart.

Salmonella positive and negative samples

The samples that were positive for the presence of *Salmonella* were compared to those that tested negative for the presence of *Salmonella* for all of the general water quality parameters and microbial parameters to determine if there were any parameters that contributed to *Salmonella* occurrence. There was a significant difference (ANOVA P-value= 0.02) between coliforms in *Salmonella* negative and *Salmonella* positive samples. There was no significant difference in *E. coli* levels between *Salmonella* negative and *Salmonella* positive samples (P = 0.11). There was also no significant difference in somatic coliphage and F+ coliphage levels for *Salmonella* positives compared to *Salmonella* negative samples (P = 0.58 and P = 0.80, respectively). The only water quality parameters that showed a significant difference when comparing *Salmonella* positives and negatives were water and air temperature (P = 0.01 and P = 0.05, respectively). There were no statistically significant differences (P-value ≥ 0.05) for *Salmonella* positive samples compared to *Salmonella* negative samples for the other physiochemical parameters of pH, turbidity, relative humidity, conductivity, salinity, and total dissolved solids (TDS).

Seasonality and Rainfall

Samples were collected during the winter months between January and April (*n* = 175), and during the summer months between June and August (*n* = 180). There were 16 positive *Salmonella* isolates (9%) recovered during the winter months and 25 positive *Salmonella* isolates (14%) that were recovered during the summer months. The average concentration of *Salmonella* during the summer months in the irrigation canals was 51 MPN/100 ml and during the winter months, the average was 10 MPN/100 ml; however, there was no statistically significant difference in the prevalence of *Salmonella* during the winter compared to the summer months. Rainfall was monitored three days prior to each sampling date (AZMET, 2018). There was only one day of sampling in February that had measurable rainfall. Thus, no comparisons could be made due to the limited rainfall data. For seasonality, higher water temperatures and higher air temperatures did have a positive correlation on microbial occurrence / survival compared to other parameters (See Table 9).

Overall Correlations in all microbial and physiochemical parameters

For the physiochemical parameters, there were strong positive correlations between water temperature and air temperature (r=0.81) and a strong positive correlation between conductivity, salinity, and total dissolved solids (r=1.00). For the microbial parameters, there was a positive correlation between coliforms and water temperature (r=0.70). There were no strong correlations (r>0.7 or r<-0.7) seen among *E. coli*, somatic coliphages, and F+ coliphages (See Table 9). However, there were slightly elevated weak positive correlations (ranging from r= 0.30 - 0.46) that were observed between *E. coli* and water temperature (r=0.46), somatic coliphages and water temperature (r=0.35), *E. coli* and coliforms (r=0.36), somatic coliphages and *E. coli* (r=0.39), air temperature with coliforms (r=0.44), air temperature with *E. coli* (r=0.36), and air temperature with somatic coliphages (r=0.36). For these correlations, the data were paired and then sorted by one parameter from low to high data values. For instance, the *E. coli* values were sorted from low to high water temperatures, then the *E. coli* levels in the top and bottom 25% of the samples sorted by water temperature were analyzed to determine if they were significantly different (two-sided t-test) for *E. coli* levels. All of these weak

correlations were significantly different when analyzed in this manner, which confirmed and strengthened the previously observed correlations (See Table 9) (i.e., correlations between *E. coli* and water temperature, somatic coliphages and water temperature, *E. coli* and coliforms, somatic coliphages and *E. coli,* and air temperature and coliforms, *E. coli*, and somatic coliphages). There were no correlations between the presence of *Salmonella* and any of the other parameters.

Salmonella positive sites

There were several sites that had a higher or more frequent recovery of *Salmonella* in irrigation water samples. The site that had the highest occurrence of *Salmonella* was site number 1, with seven positive samples out of 10 sampling trips. This site is a small canal in an urban area and it is near a main street, right behind a neighborhood. It generally had low water levels, birds (one sampling trip- observed sitting on the canal), with algae and fish. The second highest *Salmonella* positive site was 2B where four out of 10 sampling trips yielded positive *Salmonella* isolates. This canal site was in an urban area, and was a lined, lateral canal surrounded by neighborhoods. The canal usually had algae, trash and a foul smell along with low water levels. The next site that yielded the most *Salmonella* positive samples was site 11A, three of the 10 sampling trips resulted in positive isolates. Canal 11A is an unlined drainage canal in a rural area with shallow water, plants, algae, fish, and generally clear water. Canal 19 yielded three out of ten *Salmonella* positive trips. Canal 19 is a lined, lateral canal in a suburban area where birds were seen near the canal during at least two of the sampling trips along with fish, trash, and algae. Canal 8B had two *Salmonella* positive samples out of 9 sampling trips. Canal 8B is an unlined main canal in a suburban area with reports of fish, algae, plant debris, and lots of trash. Canals 7A, 13, 16A, and 20A all resulted in *Salmonella* positive isolates in two out of 10 sampling trips. These canals all had fish, algae, plant debris, and trash during sampling. Reasons these sites were *Salmonella* positive are likely because there are birds near and in the canals; also, canals with fish attract birds. Most of these canals are in urban and suburban areas where there is also more human traffic and trash. The other portion of canals that were *Salmonella*

positive were the drainage canals. When animals enter fields, they can defecate and the water in the fields that runs off into the drainage canals can transport *Salmonella* into the water.

Monte Carlo Simulation

A Monte Carlo simulation and exponential dose response model was used to determine the risk of infection from *Salmonella enterica* due to the consumption of crops or produce water irrigated with potentially contaminated water in Southern Arizona. The parameters were put into the Simulacion 4.0© program in Excel where the dose (N) was calculated and a Monte Carlo simulation was run (Varela 2003; US EPA Exposure Factors Handbook 2011). The calculated dose was then put in to the exponential model, which resulted in the probability of infection annually for the risk of *Salmonella* from irrigation water (with a concentration of 4.1 MPN/100 ml in the water) to lettuce one day after irrigation with a value of 7.1×10^{-7} (See Table 10). The same simulation was performed seven days after irrigation where *Salmonella* would be on produce for seven days (microbial die off over seven days), the probability of infection 1.6×10^{-8} annually, which is a very acceptable risk. This probability of infection is acceptable for both of the above because it is a risk lower than the EPA annual microbial limit of 1 in 10,000 (Stine et al. 2005; US EPA 2012). The high range of *Salmonella* which was 1,000 MPN/100 ml, was also tested in the same Monte Carlo Simulation on day one and day seven for the annual probability of infection which resulted in 5.1×10^{-5} and 3.8×10^{-6}, respectively. Both of these annual risks are acceptable based on EPA standards in consuming lettuce or leafy vegetables that maybe irrigated with water contaminated with *Salmonella* at an average concentration of 4.1 MPN/100ml and/or 1000MPN/100 ml at day one and day 7 post irrigation.

Exponential equation/model

P(infection)= 1- exp^(-k*N)

N= 0.000143 (4.1 MPN/100 ml, day one)

k= 3.97x10^-6

P= 1- exp (-3.97x10^-6 * (N))

Annual P infection = 7.1×10^{-7}

Discussion

The overall findings in this study suggest that the microbial quality of irrigation water in Southern Arizona is typically acceptable based on the samples collected. The quality of open irrigation canal water can change depending on many factors. This can be demonstrated by looking at the range of *Salmonella* concentration in irrigation canals which was 0.1 MPN/100 ml to 1,000 MPN/100 ml. Some factors caused increase levels of *Salmonella* at certain sampling sites.

Fecal bacterial indicators

E. coli

Generic *E. coli,* using the Colilert method, from various water irrigation sources in central California had a lower rate of 78% (199/255) positive recovery of *E. coli* (Benjamin et al., 2013) compared to the present study which had 97% (344/355). However, the average concentration of generic *E. coli* was much higher (710 CFU/100 ml arithmetic mean) (Benjamin et al., 2013) and above the EPA guidelines compared to the present study (64 MPN/100 ml arithmetic). Luo et al. (2015) sampled 10 irrigation ponds in areas of Florida and Georgia and found an overall geometric mean of generic *E. coli* of 6.26 MPN/100 ml of water, using a similar Quanti-Tray method, which was lower than the geometric mean of the present study which was 27 MPN/100 ml. In the two irrigation ponds, a total of six samples exceeded the >410 MPN/100 ml of *E. coli* limit out of 635 samples over a two year period (Luo et al., 2015). In the present study, there were 22 samples (three from drainage canals, eight from main canals, and 11 from lateral/sublateral canals) that exceeded the >410 MPN/100 ml *E. coli* limit from 355 samples in less than a one-year period. Jones et al. (2014) found *E. coli* in 16 out of 18 irrigation ponds sampled in New York; a 33% (40/123) positive recovery of *E. coli* was found. However, Jones et al. (2014) did not used the Colilert Quanti-Tray method. Colilerts are known to overestimate the number of generic *E. coli* (Rock, Gerba, and Bright, 2012, unpublished data) so this maybe why

the current study has more generic *E. coli* present. The presence of generic *E. coli* in the present study was higher (97%, 344/355) and 51 of the 53 irrigation canal sites sampled contained *E. coli*. Overall, the finding for *E. coli* were similar to those found in the studies mentioned above, some were higher and some were lower, but the overall average of *E. coli* in this study were below the FDA limits of >410 CFU/100 ml (FDA, 2018).

Coliforms

The presence of coliforms in 10 irrigation ponds in Florida and Georgia had an overall geometric mean of 10.3 CFU/100 ml of water over a one-year period (Luo et al., 2015), which was much lower than the present study which found 1,441 CFU/100 ml (geometric mean). Steele et al. (2005) in Canada examined 27 irrigation water sources located on 17 farms in Ontario and found mean values of 8,559 coliforms/100 ml of water from 494 samples collected. These levels were much higher than the present study which found an average of 1,649 CFU/100 ml total coliforms. One study in Mexico by Castro-Rosas et al. (2012) gives us an example of what can happen with the contaminated irrigation canal. The study looked at ready-to-eat (RTE) salads irrigated with untreated sewage water and found a 99% (129/130) fecal coliform recovery; the average fecal coliform concentration was 571 fecal coliforms/gram. These samples were purchased from various restaurants in Mexico in popular tourist areas which included chain, local, and small restaurants (Castro-Rosas et al., 2012).

Coliphages

Currently there are no regulations for limits on the concentration of viruses in surface waters. However, there is a regulation by the Arizona Department of Environmental Quality (ADEQ) of one virus particle/40 Liters of water in Arizona for reclaimed water used for the irrigation of food crops (ADEQ, 2016; Gerba, 2009; Gerba & Choi, 2006). Espinosa et al. (2009) conducted a study in Mexico City and found a strong correlation for the recovery of coliphages and enteroviruses (P-value=0.0182) in the same samples. Jiang et al. (2007) detected an average of 1.79 MPN of F+ coliphage /100 ml, which were much lower compared to the total arithmetic

average found in the current study which had a 59 MPN/100 ml average. A study in South Carolina looked at the presence of coliphages over a years' time from various watershed samples from 96 surface water stations (Stewart-Pullaro et al., 2006). A total of 117 surface water samples from 96 surface water stations were analyzed with an average of 52 PFU/100 ml for F-specific coliphages, which was almost the same as the present study (59 MPN/100 ml) and 313 PFU/100 ml for somatic coliphages, which was much higher than the recovery of somatic coliphage in the present study (3 MPN/100 ml). In Mexico, a study was done where 146 irrigation water samples were analyzed for coliphages in a large irrigation canal. Coliphages were detected in less than 30% of samples with an average of 141 PFU/100 ml (Gortáres-Moroyoqui et al., 2011), which is much higher than this study for either coliphages typ*E. coli*phages could possibly be a good indicator of fecal contamination in water, but there is still some uncertainty if they are also good indicators of enteric viruses.

Salmonella

There are many ways *Salmonella* can be transferred to produce in fields, but one of the easiest routes is via irrigation water (Steele & Odumeru, 2004). A study in New York tested various types of surface waters for the prevalence of *Salmonella*. Out of 18 sites for a total of 123 samples collected, there was a 43% *Salmonella* positive recovery (Jones et al., 2014), which is much higher than the present study (11.6%). Another study in Georgia by Haley et al. (2009) looked at the prevalence of *Salmonella* along two large rivers with samples collected monthly over a years' time and found 79.2% (57/72) of the samples were positive for *Salmonella* (Haley et al., 2009). Another study in Florida and Georgia looked at the prevalence of *Salmonella* monthly from 10 irrigation ponds (Luo et al., 2015) and recovered 28.2% (179/635) *Salmonella* positive isolates, which was almost double the number of positives found in the present study (11.6%, 41/355). In the central California coast region, only 6% (6/96) of *Salmonella* positive isolates were recovered from various irrigation water sources (Benjamin et al., 2013), which was almost half of the *Salmonella* positive isolates found in this study. There have not been many studies that have looked at the prevalence of *Salmonella* in irrigation waters. Of the studies mentioned, there were high and low recoveries of *Salmonella* and the results of this

study fell somewhere in the lower spectrum. This indicates that Arizona might have a lower and safer prevalence of *Salmonella* in irrigation water and based on the total average MPN concentration (4.10 MPN/100 ml) and risk assessment, the risk is low and acceptable.

Historical data in Southern Arizona

This section is comparing historical data to the current data from the same sites of irrigation water canals in Southern Arizona. In a study in which samples were collected from 2001 and 2003, 28.9% (30/112) *Salmonella* positive isolates were recovered (Kayed, 2004) which is more than double the results from this study (11.6%). The same study also looked at the average concentrations of *E. coli* and total coliforms which were 44 MPN/100 ml (113 samples, arithmetic mean) and 9,885 MPN/100 ml (113 samples, arithmetic mean), respectively (Kayed, 2004). The average arithmetic concentration of *E. coli* (64 MPN/100 ml) in the present study is similar and for coliforms (1,649 MPN/100 ml), the average was much lower in the present study. Another study from 2011 to 2014 sampled irrigation water canals in Southern Arizona and recovered 6.1% (68/1120) *Salmonella* positive isolates with a geometric mean of 55 MPN/sample from biofilms in areas of irrigation canals such as irrigation pumps, sprinkler pipes, and other irrigation canal components (Bright et al., 2014, unpublished data). This recovery of *Salmonella* is much lower than what was found in the present sampling study. However, the geometric mean per sample was higher in this study (55 MPN/sample) than in the current study (0.02 MPN/100 ml). Another study which collected irrigation water samples from 2010 to 2015 in the same areas in Southern Arizona recovered 14% (33/236) *Salmonella* positive isolates as well as 88% (208/236) positive *E. coli* samples (Bright et al., 2015, unpublished data). The recovery of *Salmonella* positives was higher in this study and the incidence of *E. coli* positives were higher in the present study (97%). In 2016 to 2017, irrigation water samples were collected from Southern Arizona and 2.7% (8/297) *Salmonella* positive isolates were detected (Bright et al., 2017, unpublished data), which is much lower than the present study. There is a lot of variation in the results from historical data to the current data. There are highs and lows, but it appears that *Salmonella* was higher in the older studies compared to the current study. This could be an indication that our water quality is improving due to awareness of the

potential risks of foodborne illnesses, improvement in technology, and learning sources of pathogens such as *Salmonella*.

Conclusions

In conclusion, the current study shows that *Salmonella* occurs in some areas more often than others and ranges in concentrations from 0.01 to 1,000 MPN/100 ml. There were 11.6% confirmed *Salmonella* positive isolates from 355 samples collected with an average concentration of 4.1 MPN/100 ml. Also, based on the risk assessment and Monte Carlo simulation performed using this data, the risk of contracting *Salmonella* from lettuce irrigated with waters from this region are below the annual probability of infection microbial limit of 1 in 10,000, the EPA standard. The prevalence of *E. coli* (97%, 64 MPN/100 ml average), coliforms (100%, 1,648 MPN/100 ml average), and somatic and F+ coliphages (61%, 3 MPN/100 ml average and 35%, 60 MPN/100 ml average, respectively) could indicate human or animal fecal contamination is occurring in the irrigation canals. Some of these including *Salmonella* were slightly higher in drainage canals. This could be since the drainage canals collect the overflow of water from irrigation fields. These organisms could persist in the soil and then can travel in the water into the drainage canals. These fecal indicator organisms could also indicate other bacterial or viral pathogens that might also potentially be present. The sites where *Salmonella* occurred most frequently were in areas with ducks, birds, or fish (which are food for birds and other animals) and drainage canals. Statistical findings show that *Salmonella* does not significantly correlate with any of the microbial or general water quality parameters. Overall, this study shows that irrigation canals in Southern Arizona are relatively low-risk; however, nothing is risk free and more sampling would help to strengthen the risk characteristics.

List of Tables

TABLE 1. Overall microbial positives and concentrations found from all irrigation canal sites.

Total Coliforms		***E. coli***		**Somatic Coliphage**		**F+ Coliphage**		***Salmonella***	
355/355 (100%)		344/355 (97%)		127/210 (60.5%)		62/175 (35.4%)		41/355 (11.6%)	
Arith*	Geo*	Arith	Geo	Arith	Geo	Arith	Geo	Arith	Geo
1648.7	1440.5	64.1	26.6	3.2	2.3	58.5	11.6	4.0	0.02

*Arith = arithmetic mean, *Geo = geometric mean

TABLE 2. Salmonella confirmed positive isolates based on site location.

Site#	**Canal Type**	**Canal Characteristics**	**Canal area**	***Salmonella* positive samples**	**Total Sampling trips per site**
1	Lateral Canal	LACS*	Urban	7	10
1B	Lateral Canal	Unlined	Urban	0	1
2A	Lateral Canal	LACS	Urban	0	9
2B	Lateral Canal	Lined	Urban	4	10

3A	Lateral Canal	Lined	Urban	0	10
3B	Lateral Canal	Lined	Urban	0	1
3C	Lateral Canal	Lined	Urban	0	1
4A	Main Canal	Unlined	Urban	0	9
4B	Main Canal	Unlined	Urban	1	10
5A	Main Canal	Unlined	Urban	1	10
5B	Main Canal	Unlined	Urban	1	10
6A	Main Canal	Unlined	Rural	0	10
6B	Main Canal	LACS	Rural	0	10
7A	Main Canal	Unlined	Suburban	2	10
7B	Drainage	Unlined	Suburban	0	8
8A	Main Canal	Unlined	Suburban	1	10
8B	Main Canal	Unlined	Suburban	2	9
9	Main Canal	Unlined	Urban	1	10
10A	Drainage	LACS	Rural	0	9
10B	Main Canal	LACS	Rural	0	9
11A	Drainage	Unlined	Rural	3	10
11B	Drainage	Unlined	Rural	1	3
12	Main Canal	LACS	Rural	0	10
12B	Lateral Canal	Lined	Suburban	0	1
13	Drainage	Unlined	Suburban	2	10
14A	Lateral Canal	Lined	Suburban	0	10
14B	Lateral Canal	Lined	Suburban	0	1
15A	SLC**	Lined	Suburban	1	9
15B	SLC**	Lined	Suburban	1	9
16A	Main Canal	LACS	Rural	2	10
16B	Main Canal	Unlined	Rural	0	10
17	Drainage	Unlined	Suburban	1	9
18A	SLC**	LACS	Rural	1	10
18B	SLC**	Unlined	Rural	0	2
18C	SLC**	Unlined	Rural	0	1
19	Lateral Canal	Lined	Suburban	3	10
19B	Lateral Canal	Lined	Suburban	0	1
20A	Main Canal	Unlined	Rural	2	10
20B	Main Canal	Unlined	Rural	1	10
21	SLC**	Unlined	Rural	0	9
21B	SLC**	Lined	Rural	0	1
22A	Lateral Canal	Lined	Suburban	0	8

22B	Lateral Canal	Lined	Suburban	0	1
23A	Lateral Canal	Unlined	Rural	0	9
23B	Lateral Canal	Unlined	Rural	0	9
23C	SLC**	Unlined	Rural	1	8
24A	Lateral Canal	Lined	Rural	0	1
24B	Lateral Canal	Lined	Rural	0	2
M1	Lateral Canal	Lined	Rural	0	1
M2	Lateral Canal	LACS*	Rural	1	1
M3	Lateral Canal	Lined	Suburban	1	1
M4	Drainage	Lined	Rural	0	1
M5	Lateral Canal	Lined	Suburban	0	1

*LACS- Lined at collection site. **SLC- Sublateral canal

TABLE 3. Average water and air parameters from irrigation canals.

Site #	Canal Type	Lined/ Unlined	Water Temp (°C)	pH	Turb. (NTU)	Relative Humidity %	Cond. (µS/cm)	Salinity (ppm)	Total Dissolved Solids (ppm)	Air Temp (°C)
1	Lateral	LACS*	24.5	8.3	3.5	25	1107	539	778	33.6
1B	Lateral	Unlined	14.4	8.3	8.5	37	1216	594	864	15.2
2A	Lateral	LACS	24.7	8.3	3.8	23	1114	550	792	34.4
2B	Lateral	Lined	25.7	8.5	4.2	24	1101	542	781	33.9
3A	Lateral	Lined	24.3	8.3	3.4	24	1104	545	784	34.2
3B	Lateral	Lined	20.7	8.1	12.4	13	1067	524	756	37.1
3C	Lateral	Lined	13.7	8.1	3.8	37	1214	590	858	16.0
4A	Main	Unlined	24.6	8.2	3.9	22	1113	546	788	35.3
4B	Main	Unlined	23.9	8.1	3.7	25	1117	552	794	32.8
5A	Main	Unlined	24.0	8.2	4.2	23	1123	554	796	35.1
5B	Main	Unlined	24.0	8.3	4.1	22	1123	553	797	35.5
6A	Main	Unlined	23.1	8.3	6.4	31	1132	558	803	30.6
6B	Main	LACS	23.1	8.2	5.0	30	1130	557	802	31.3
7A	Main	Unlined	23.3	8.2	6.1	28	1120	552	794	32.1
7B	Drainage	Unlined	24.7	8.1	4.5	27	1122	554	796	33.3
8A	Main	Unlined	23.1	8.2	3.9	31	1123	554	798	31.8
8B	Main	Unlined	24.1	8.2	4.0	29	1114	550	791	32.7
9	Main	Unlined	23.5	8.3	2.9	28	1131	557	804	31.9
10A	Drainage	LACS	26.3	7.5	1.5	27	2741	1416	1943	33.2
10B	Main	LACS	24.0	8.1	3.3	27	1127	555	795	33.4
11A	Drainage	Unlined	25.2	7.6	2.5	30	2440	1252	1728	30.5
11B	Drainage	Unlined	24.9	7.6	2.1	34	2370	1210	1683	25.3
12	Main	LACS	22.8	8.1	4.0	30	1131	557	803	29.9

12B	Lateral	Lined	13.0	8.3	3.1	46	1198	582	850	14.1
13	Drainage	Unlined	22.3	7.9	14.6	33	3010	1585	2175	29.7
14 A	Lateral	Lined	23.0	8.2	3.3	32	1127	554	800	30.1
14B	Lateral	Lined	14.8	8.2	1.6	52	1115	543	791	17.0
15 A	Sublateral	Lined	22.1	8.0	2.4	34	1116	551	793	28.9
15B	Sublateral	Lined	22.3	8.1	7.1	30	1119	551	793.0	29.8
16 A	Main	LACS	22.1	8.2	9.9	38	1178	581	835	26.4
16B	Main	Unlined	22.2	8.2	9.1	36	1171	575	826	26.8
17	Drainage	Unlined	24.7	7.7	5.0	40	2106	1052	1494	27.1
18 A	Sublateral	LACS	22.0	8.2	8.9	41	1162	571	823	26.5
18B	Sublateral	Unlined	19.0	8.0	4.1	57	1878	1310	1329	13.9
18C	Sublateral	Unlined	11.7	8.3	3.7	52	1289	625	918	12.7
19	Lateral	Lined	22.0	8.2	9.3	38	1157	570	821	26.2
19B	Lateral	Lined	11.7	8.4	3.7	45	1260	610	894	14.5
20 A	Main	Unlined	22.0	8.2	16.5	41	1158	571	822	25.6
20B	Main	Unlined	22.0	8.2	14.7	38	1159	570	823	26.7
21	Sublateral	Unlined	20.2	8.1	9.1	52	1146	562	814	22.4
21B	Sublateral	Lined	11.3	8.5	3.1	57	1294	627	919	9.8
22 A	Lateral	Lined	24.0	8.1	12.0	43	1118	553	793	27.0
22B	Lateral	Lined	26.4	8.8	2.7	9	1132	561	804	40.0
23 A	Lateral	Unlined	22.7	8.2	12.7	45	1153	569	817	26.2
23B	Lateral	Unlined	22.8	8.2	12.2	45	1139	558	807	27.4
23C	Sublateral	Unlined	22.0	8.1	11.3	48	1124	552	797	25.5

24A	Lateral	Lined	12.6	8.3	1.7	48	1202	582	853	15.8
24B	Lateral	Lined	14.0	8.4	1.4	42	1220	595	867	19.0
M1	Lateral	Lined	29.3	8.2	1.4	18	1530	767	1080	38.8
M2	Lateral	LACS	31.7	9.1	1.9	16	1051	521	746	38.1
M3	Lateral	Lined	32.0	8.8	4.1	19	976	481	692	40.2
M4	Drainage	Lined	31.1	8.4	7.1	13	1456	730	1030	41.1
M5	Lateral	Lined	31.8	8.6	4.8	17	1005	497	714	39.7

*LACS – lined at collection site

TABLE 4. Average concentrations of indicator organisms found in irrigation canal sites.

Site #	Total Coliforms (MPN/100ml)		*E. coli* (MPN/100 ml)		Somatic Coliphage (MPN/100 ml)		F+ Coliphage (MPN/100 ml)		*Salmonella* (MPN/100ml)	
	arithmetic	geometric	arithmetic	geometric	arithmetic	geometric	arithmetic	geometric	arithmetic	geometric
1	2093.78	1897.98	378.60	52.19	1.28	1.14	3.60	2.24	14.02	0.63
1B	920.80	920.80	23.10	23.10	1.00	1.00	ND	ND	0.01	0.01
2A	1952.51	1532.61	380.79	83.83	4.58	4.15	5.08	2.12	0.01	0.01
2B	1808.98	1359.27	88.66	34.85	1.65	1.37	195.04	6.08	0.32	0.05
3A	1944.76	1505.01	23.71	19.23	1.63	1.35	2.90	1.51	0.01	0.01
3B	547.50	547.50	7.50	7.50	ND	ND	ND	ND	0.01	0.01
3C	228.20	228.20	18.70	18.70	6.30	6.30	ND	ND	0.01	0.01
4A	1419.98	903.08	18.97	13.69	5.20	2.22	1.78	1.33	0.01	0.01
4B	1333.71	664.36	39.92	18.29	4.08	1.83	2.64	1.44	10.01	0.03
5A	1347.12	764.47	29.43	20.90	10.05	4.98	1.34	1.15	0.02	0.01
5B	1358.15	774.96	29.23	20.42	8.55	2.84	3.10	1.51	0.02	0.01
6A	1533.74	882.96	17.73	11.16	3.07	2.39	5.74	1.79	0.01	0.01
6B	1385.33	787.89	14.44	10.02	2.33	1.95	3.26	1.53	0.01	0.01
7A	1529.47	1046.15	45.58	16.95	2.92	2.00	2.64	1.76	10.11	0.04
7B	1859.51	1262.10	107.66	25.49	3.92	3.27	8.78	2.20	0.01	0.01
8A	1427.73	928.77	85.79	29.82	4.42	2.96	2.44	1.64	0.11	0.02
8B	1582.82	1217.65	94.42	38.63	6.62	4.25	5.18	2.76	2.23	0.05

9	1389.50	873.53	31.18	15.29	4.35	2.40	2.38	1.60	1.01	0.02
10A	2419.60	2419.60	7.21	3.67	0.92	0.92	2.18	1.37	0.01	0.01
10B	1575.70	1180.98	105.73	32.70	9.00	4.69	2.62	1.80	0.01	0.01
11A	2419.60	2419.60	15.91	7.44	4.63	1.55	2.64	1.44	0.31	0.04
11B	2419.60	2419.60	7.67	3.31	0.90	0.90	ND	ND	0.04	0.02
12	1430.20	945.77	81.34	17.22	13.13	4.91	2.42	1.41	0.01	0.01
12B	365.40	365.40	6.30	6.30	1.00	1.00	ND	ND	0.01	0.01
13	2419.60	2419.60	313.70	42.63	0.90	0.90	10.00	2.06	0.12	0.02
14A	1624.73	1250.86	372.72	39.15	4.62	1.98	15.10	2.16	0.01	0.01
14B	1119.90	1119.90	5.20	5.20	2.00	2.00	0.90	0.90	0.01	0.01
15A	1840.64	1563.00	112.26	28.11	4.94	2.57	6.30	2.29	0.02	0.01
15B	1860.50	1593.86	140.72	41.45	3.82	2.01	14.46	2.29	111.12	0.04
16A	1638.29	1281.65	111.95	15.94	4.02	1.76	80.68	3.05	1.11	0.03
16B	1576.22	1167.55	144.70	22.01	5.28	2.47	23.66	2.42	0.01	0.01
17	2419.60	2419.60	100.43	28.66	1.02	2.02	9.64	122.20	1.12	0.02
18A	1818.59	1551.34	44.39	28.38	1.62	1.32	23.64	2.37	0.11	0.02
18B	1347.55	816.46	15.40	15.39	0.90	0.90	ND	ND	0.01	0.01
18C	866.40	866.40	6.30	6.30	1.00	1.00	ND	ND	0.01	0.01
19	1848.37	1457.64	27.11	12.89	1.38	1.85	12.94	64.70	0.14	0.03
19B	344.80	344.80	7.40	7.40	3.00	3.00	ND	ND	0.01	0.01

20A	1603.38	773.32	22.97	7.45	1.82	1.54	49.26	5.93	1.02	0.03
20B	1821.73	1122.42	20.52	8.19	1.63	1.35	127.74	6.13	0.02	0.01
21	2085.39	1633.46	119.36	34.42	0.68	1.76	126.08	25.09	0.01	0.01
21B	172.50	172.50	0.90	0.90	1.00	1.00	ND	ND	0.01	0.01
22A	2068.03	1946.19	19.96	8.48	0.95	0.95	11.44	3.16	0.01	0.01
22B	2419.60	2419.60	0.90	0.90	ND	ND	ND	ND	0.01	0.01
23A	2015.13	1707.25	9.46	5.21	0.90	0.90	492.40	9.31	0.01	0.01
23B	2171.42	1819.45	8.42	5.23	0.96	0.96	744.40	18.71	0.01	0.01
23C	2087.44	1723.79	39.15	12.12	1.16	1.10	17.62	2.23	0.13	0.02
24A	248.90	248.90	8.60	8.60	2.00	2.00	ND	ND	0.01	0.01
24B	1573.30	1326.29	1.45	1.34	0.90	0.90	ND	ND	0.01	0.01
M1	2419.60	2419.60	11.80	11.80	ND	ND	ND	ND	0.01	0.01
M2	2419.60	2419.60	18.50	18.50	ND	ND	ND	ND	10.00	10.00
M3	2419.60	2419.60	30.50	30.50	ND	ND	ND	ND	1.00	1.00
M4	2419.60	2419.60	15.80	15.80	ND	ND	ND	ND	0.01	0.01
M5	2419.60	2419.60	6.20	6.20	ND	ND	ND	ND	0.01	0.01

ND= Not Determined

TABLE 5. Average Water quality parameters for primary irrigation canals and return flow canals.

Canal Type	Water Temp (°C)	pH	Turbidity (NTU)	Relative Humidity (%)	Conductivity (µS/cm)	Salinity (ppm)	Total Dissolved Solids (ppm)	Air Temp (°C)
Main	23.2[ab]	8.2[a]	6.4[a]	29.9[a]	1134.4[a]	558.8[a]	804.4[a]	31.1[a]
Lateral and Sublateral	21.0[b]	8.3[b]	5.5[a]	35.6[a]	1181.1[a]	592.5[a]	837.6[a]	26.3[b]
Drainage	25.6[a]	7.8[c]	5.3[a]	29.1[a]	2177.9[b]	1114[b]	1549.9[b]	31.5[ab]

a,b,c = Different letters indicate a significant difference (P-value ≤ 0.05)

TABLE 6. Microbial Water Content in primary irrigation canals and return flow canals.

Microbial Assay (MPN/100 ml)	Type of Average	Canal Type		
		Main	Lateral / Sublateral	Drainage
Total Coliforms (Colilert®)	Arithmetic Mean	1497.1[a]	1568.5[a]	2339.6[b]
	Geometric Mean	1491.7[a]	1263.9[a]	2330.3[b]
***Escherichia coli* (Colilert®)**	Arithmetic Mean	55.8[a]	64.4[a]	81.1[a]
	Geometric Mean	42.8[a]	19.5[b]	33.2[ab]
Somatic Coliphages (FastPhage®)	Arithmetic Mean	5.4[a]	2.4 [b]	2.04[b]
	Geometric Mean	4.6[a]	1.6[b]	1.5[b]
F+ Coliphages (FastPhage®)	Arithmetic Mean	19.8[a]	111.5[a]	6.6[a]
	Geometric Mean	5.9[ab]	20.14[a]	5.5[b]
Salmonella	Arithmetic Mean	1.6[a]	4.6[a]	0.23[a]
	Geometric Mean	0.1[a]	0.04[a]	0.1[a]
Salmonella positive samples	N/A	14/157 (9%)	20/148 (13.5%)	7 / 50 (14%)

[a,b,c] = Different letters within the same row indicate a significant difference (P-value ≤ 0.05)

TABLE 7. Average water quality parameters in Lined and Unlined irrigation canals.

Canal type	Water Temp(°C)	pH	Turbidity (NTU)	Relative Humidity(%)	Conductivity (µS/cm)	Salinity (ppm)	Total Dissolved Solids (ppm)	Air Temp (°C)
Lined	22.3	8.3*	4.6*	31.0%	1215.6	600.0	861.7	29.0
Unlined	22.3	8.1*	7.1*	35.5%	1399.0	713.1	994.1	27.8

*Indicates the P-value ≤ 0.05

TABLE 8. Microbial Water Content in Lined and Unlined irrigation canals.

Canal type	Total Coliforms (MPN/100ml)		*E. coli* (MPN/100 ml)		Somatic Coliphage (MPN/100ml)		F + Coliphage (MPN/100ml)		*Salmonella*		
	arith	Geo	arith	geo	arith	geo	arith	geo	arith	geo	Confirmed positive isolates
Lined	1600.97	1290.93	70.67	22.09	2.49	2.39	13.19	7.73	4.76	0.04	12.7% (20/157)
Unlined	1706.48	1644.64	56.12	33.16	3.17	2.26	68.31	11.72	1.10	0.07	10.6% (21/198)
Total Average	1653.73	1440.54	63.40	26.55	3.22	2.32	52.94	9.74	4.02	0.02	11.6%(41/355)

arith = arithmetic mean.
geo = geometric mean

TABLE 9. Total Correlations for Microbial and Physiochemical parameters

	Rainfall (Y/N)	Rainfall (in.)	Water Temp (°C)	pH	Turbidity (NTU)	Relative Humidity (%)	Conductivity (µS/cm)	Salinity (ppm)	Total Dissolved Solids	Air Temp (°C)	Total Coliforms	*E. coli* (MPN/100 ml)	Salmonella MPN/100 ml	S Total Coliphage	F+ Total Coliphage
Rainfall (Y/N)	1.00														
Rainfall (in.)	1.00	1.00													
Water Temp (°C)	-0.35	-0.35	1.00												
pH	0.11	0.11	-0.23	1.00											
Turbidity (NTU)	-0.03	-0.03	0.08	-0.04	1.00										
Relative Humidity (%)	0.06	0.06	-0.05	0.04	0.01	1.00									
Conductivity (µS/cm)	0.00	0.00	0.03	-0.57	0.00	-0.04	1.00								
Salinity (ppm)	0.00	0.00	0.04	-0.57	0.00	-0.04	1.00	1.00							
Total Dissolved Solids (ppm)	0.01	0.01	0.03	-0.57	-0.01	-0.04	1.00	1.00	1.00						
Air Temp (°C)	-0.41	-0.41	0.81	-0.12	0.00	-0.11	-0.07	-0.06	-0.07	1.00					
Total Coliforms (MPN/100 ml)	-0.05	-0.05	0.70	-0.25	0.15	0.05	0.19	0.20	0.19	0.44	1.00				
E. coli (MPN/100 ml)	-0.19	-0.19	0.46	-0.11	0.06	0.09	-0.11	-0.10	-0.11	0.36	0.36	1.00			
Salmonella MPN/100 ml	-0.07	-0.07	0.15	0.04	0.02	0.00	0.00	0.00	0.00	0.12	0.12	0.12	1.00		
S Total Coliphage (MPN/100	-0.19	-0.19	0.35	-0.04	-0.09	0.02	-0.22	-0.21	-0.22	0.36	0.13	0.39	0.00	1.00	
F+ Total Coliphage (MPN/100	-0.11	-0.11	0.00	0.08	-0.02	-0.10	-0.05	-0.05	-0.06	0.10	0.13	-0.02	-0.04	-0.11	1.00

Highlighted boxes: Yellow indicates a weak negative correlation ($r < 0.60$), Green indicates a strong positive correlation ($r > 0.70$), and Blue indicates a weak positive correlation ($r = 0.30$-0.46)

TABLE 10. Risk Assessment Information

Exponential model [P(infection)=1-exp(-k*dose)]

Agent	Best fit model	Optimized parameter	LD50/ ID50	Host type	Agent strain	Route	# of doses	Dose units	Response	Reference
Salmonella serotype newport: Dose Response Models	exponential	k=3.97E-06	1.74E+05	human	*Salmonella newport*	oral	3	CFU	infection	McCullough & Eisele, 1951

Parameter (Point estimate)	Assumption
Bacterial die off rate (Stine et al., 2005)	0.35 logs/day
Transfer of bacteria from irrigation water to lettuce (Stine et al., 2005)	0.00011%

Parameter (Stochastic)	Assumptions (mean, SD, range)		
Salmonella concentration (MPN/100 ml) (Gerba, 2018)	4.1	53.8	0.1-1000
Ingestion rate- Lettuce (g/day) (EPA, 2018)	14.3	1.13	0-429
Ingestion rate Leafy vegetables (g/day) (EPA, 2018)	34.4	1.65	0-751

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