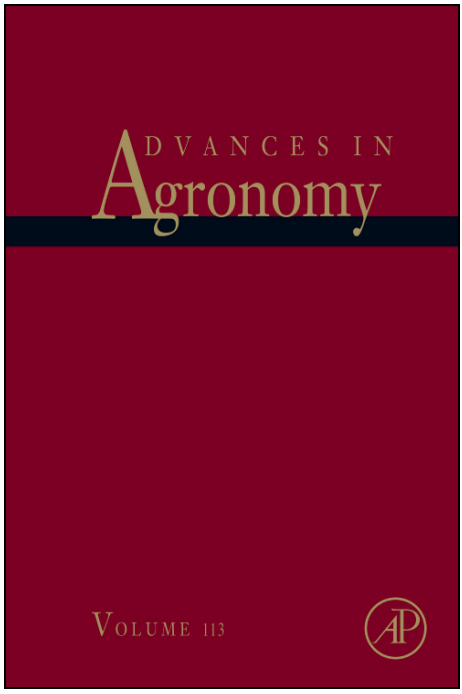

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CHAPTER TWO

Irrigation Waters as a Source of Pathogenic Microorganisms in Produce: A Review

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Contents

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Abstract

There is increasing evidence that consumption of raw fresh produce is a major factor contributing to human gastrointestinal illness. A wide variety of pathogens contribute to food-borne illnesses, including bacteria (e.g., *Salmonella*, pathogenic *Escherichia coli*), protozoa (e.g., *Cryptosporidium*, *Giardia*), and viruses (e.g., noroviruses). Large-scale production of produce typically requires some form of irrigation during the growing season. There is a rapidly growing body of research documenting and elucidating the pathways of produce contamination by water-borne pathogens. However, many gaps still exist in our knowledge and understanding. The purpose of this review is to provide a comprehensive approach to the issue, including the most recent research. Topics covered include: temporal and spatial variability, and regional differences, in pathogen and indicator organism concentrations in water; direct and circumstantial evidence for contaminated water as a source of food-borne pathogens; fate and transport of pathogens and indicator organisms in irrigation systems, and the role of environmental microbial reservoirs; and current standards for irrigation water quality, and risk assessment. A concerted effort by researchers and practitioners is needed to maintain food safety of fresh produce in an increasingly intensive food production system and limited and declining irrigation water resources.

1. Introduction

There is increasing evidence that consumption of raw fresh produce is a major factor contributing to human gastrointestinal illness, due to the potential for contamination with pathogenic microorganisms. Multiple surveys have been performed to determine the local prevalence of pathogenic microorganisms on fruit and vegetables. Several recent books summarized results of these surveys (Fan *et al.*, 2009; Sapers *et al.*, 2009; Warriner *et al.*, 2009). The list of pathogens of interest includes bacteria *Campylobacter* spp., enterohemorrhagic *Escherichia coli* (e.g., *E. coli* O157: H7), enterotoxigenic *Staphylococcus aureus*, enterotoxigenic *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, protozoa *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Giardia* spp., *Entamoeba histolytica*, helminths such as *Ascaris* spp., and viruses, in particular, adenoviruses, enteroviruses, noroviruses, and rotaviruses. Incidence of foodborne pathogens on fruits and vegetables varies by region and can be extremely high in some developing countries. However, substantial

outbreaks continually occur in developed countries. The produce related illnesses cost USA up to $39 billion annually (Scharff, 2009).

Large-scale production of produce typically requires some form of irrigation during the growing season. Consequently, there is a rapidly growing body of research documenting and elucidating the pathways of produce contamination by water-borne pathogens. Excellent reviews by Steele and Odumeru (2004) and Gerba (2009) have recently been published. However, many gaps exist in our knowledge and understanding. Recent Food and Agriculture Organization and World Health Organization-sponsored workshops have concluded that the role of contaminated water used in the production of vegetable crops as a vector for the transmission of these pathogens to humans is not clear (FAO/WHO (Food and Agriculture Organization of the United Nations, World Health Organization), 2008). This review builds upon these previous reviews; however our intent is to provide a more comprehensive approach to the issue as well as to include the most recent research.

No databases on microbial quality of irrigation water have been compiled to date. However, increasing evidence of contamination of produce from irrigation water and increasing scarcity in water resources leave little doubt about the need to pay more attention to the fate and transport of pathogens in irrigation waters. Sources of irrigation water can be generally ranked by the microbial contamination hazard (Leifert *et al.*, 2008): in order of increasing risk these are potable or rain water, groundwater from deep wells, groundwater from shallow wells, surface water, and finally raw or inadequately treated wastewater. In many countries, surface waters are the predominant source for irrigation. In the USA for example, the amount of irrigation water increased from 1.076×10^{11} in 2003 to 1.131×10^{11} m^3 in 2008. The number of US farms using only groundwater decreased by 9.2%, and the number of farms using only surface water increased by 6.3% (USDA-NASS, 2008). Farms using only surface water were applying 51% more water than farms that used only groundwater for irrigation. The shift to surface water use has coincided with an increase in the popularity of small farms marketing directly to consumers via farmers markets or Community Supported Agricultures (CSAs), resulting in a decrease in the average area devoted to vegetable production per farm.

A water source that is increasingly used in the USA is treated municipal wastewater. The two states producing the most treated wastewater, Florida and California, report a reuse flow of more than 1.2 billion gallons/day (WateReuse Foundation, 2006), but use of this water for food crop irrigation occurs only on a very limited scale. At this time, there are no federal regulations governing the use of municipal wastewater to irrigate crops. Nineteen USA states regulate the use of wastewater in crop production, but these regulations vary widely. Some states require very stringent treatment of effluents to reduce the concentration of pathogens to acceptable levels

prior to irrigation, while others utilize site limitations and restriction of crop utilization to allow time for pathogens to decrease to acceptable levels (National Research Council (NRC), 1996).

The objective of this paper is to review the recent research in water quality pertinent to microbiological contamination of produce from irrigation waters, and highlight the relevant information on monitoring, regulation, and control of the microbiological quality of irrigation water. While the types of irrigation systems used in produce farming vary widely (USDA-NASS, 2008), they are usually complex enough to create an ecological environment with multiple potential sources of pathogens for a particular source of water and with potential reservoirs of microorganisms including pathogenic species and strains. Both point and nonpoint sources of microorganisms affect water quality in the sources of irrigation waters. Most sources are affected by weather patterns, presence of animals, water management, and agricultural practices. The site-specific differences in fate and transport of pathogen and indicator organisms in irrigation waters make it imperative to identify the risks of produce contamination for the specific site as related to the specific type of produce, and specific irrigation management. This information is the key to establishing realistic and meaningful guidelines on microbial quality of irrigation water.

2. Concentrations of Microbial Pathogens and Indicator Organisms in Irrigation Waters

There is a substantial data base available on microbial water quality of surface waters throughout the USA and other countries based on indicator organisms. However, this information is of limited value for estimating risk for produce contamination due to deficiencies in location, timing and/or frequency of sampling. There is very little data on prevalence of specific pathogens. Although reports on the microbial contamination of irrigation water sources are available, these have mostly been conducted "after the fact" subsequent to an outbreak. In addition, surface waters used for irrigation are monitored much less intensively than drinking or recreation water, and not necessarily during periods of peak usage (e.g., during droughts). Note that process water, that is, water used in crop management but not intended for irrigation, such as water used for application of pesticides or cleaning spray equipment, is rarely (if ever) monitored. Even when irrigation water is monitored, indicator organisms rather than actual pathogens are measured in the vast majority of cases. Indicator organisms have been selected mainly to indicate the potentially occurring fecal contamination rather than presence or concentration level of any specific pathogen. The major indicator organisms are *E. coli*, fecal streptococci, and enterococci;

other groups of organisms have been recommended (*Bacteroides*, *E. coli* specific phages), but none have been widely adopted (Ashbolt *et al.*, 2001).

A comprehensive survey of microbial contamination levels in irrigation water has not yet been compiled for the USA (Stoeckel, 2009) or for any other country. We are not aware of any regular reporting on microbial quality of irrigation waters anywhere in the world. This is due, in part, to the cost of extensive sampling. In addition, producers/growers who have begun to collect data on microbial water quality may be reluctant to share these data (Suslow, 2010). Available survey data do, however, show the potential importance of pathogens in irrigation water. Thurston-Enriquez *et al.* (2002) studied occurrence of human pathogenic parasites in irrigation waters used for food crops in the United States and several Central American countries. They found that 28% of the irrigation water samples tested positive for microsporidia, 60% tested positive for *Giardia* cysts, and 36% tested positive for *Cryptosporidium* oocysts. Duffy *et al.* (2005) observed *Salmonella* in 9% of irrigation waters analyzed in Texas. A large survey of USA groundwater found that 11% of sites were positive for *Cryptosporidium*, *Giardia*, or both (Moulton-Hancock *et al.*, 2000). Close *et al.* (2008) demonstrated that intensive dairying and border-strip irrigation resulted in leaching of *E. coli* and *Campylobacter* to shallow groundwater; *E. coli* and *Campylobacter* were detected in 75% and 12% of samples, respectively. Chigor *et al.* (2010) isolated *E. coli* O157 from 2% of all samples from the river in northern Nigeria used for large-scale irrigation. The prevalence of *E. coli* O157:H7 and *Salmonella* in surface waters of Southern Alberta were 1% and 6%, respectively (Johnson *et al.*, 2003). In the same region of Southern Alberta, *E. coli* O157:H7 was isolated in 2% of 1608 samples of the surface water supplies over a 2-year period (Gannon *et al.*, 2004). Eight percent of the irrigation water samples collected from six irrigation districts in Alberta, Canada contained >100 fecal coliform/100 ml (Cross, 1997). *Salmonella* were detected in 6% of surface water samples in Greece (Arvanitidou *et al.*, 1997). In a survey of private wells in the Netherlands, Schets *et al.* (2005) found that 11% of the samples contained fecal indicators, while *E. coli* O157:H7 was isolated from 3% of the samples.

Untreated domestic wastewater contains consistently high concentrations of indicator bacteria as well as pathogens. Kay *et al.* (2008) reported total coliform counts averaging 7.6×10^{10} 100 mL^{-1} in untreated wastewater, while *Cryptosporidium* and *Salmonella* have been reported at average concentrations of 2.6–3.2 $\times$ 10^2/100 mL and 2.7 $\times$ 10^2/100 mL (Howard *et al.*, 2004; Rose *et al.*, 2001) in untreated water samples. Modern treatment methods have been shown to be effective in removal of pathogens to below limits of detection in domestic wastewater. However, research results indicate that tertiary water treatment, including final disinfection using UV light and/or chlorination, is necessary to ensure maximum removal of enteric bacteria, protozoans, and viruses (Al-Sa'ed, 2007; Gerba and Smith, 2005).

The databases on surface and groundwater quality that are available do not necessarily reflect the microbial quality of irrigation water, and may be biased toward contaminated samples because the intensive monitoring is usually conducted at the sites where extensive contamination has been known to occur (Stoeckel, 2009). As previously noted, these databases consist almost exclusively of information on fecal indicator organisms rather than on specific pathogens. Furthermore, though rare reports of correlations between indicator organisms and pathogens exist in the literature (e.g., Payment and Locas, 2010; Wilkes *et al.*, 2009), it is widely recognized that indicator organisms are poor predictors of the potential for water to cause gastrointestinal illness (Alonso *et al.*, 2006; Duris *et al.*, 2009; Harwood *et al.*, 2005; Shelton *et al;* 2011). Consequently, interpretation of data on indicator organisms in terms of concentrations of pathogens remains problematic.

2.1. Regional and local differences

Developing countries usually report much higher levels of pathogens in irrigation water than developed countries (Thurston-Enriquez *et al.*, 2002). In developing countries, untreated raw wastewater is often used for produce irrigation. Wastewater irrigation provides a quarter of all vegetables produced in Pakistan. In most parts of Sub-Saharan Africa, irrigated urban and peri-urban farming with highly polluted water sources contributes 60–100% of the perishable vegetables sold in most cities (Scott *et al.*, 2004). Fecal indicator concentrations in such waters can reach levels typical for manure and feces. Singh *et al.* (2010) found concentrations of fecal coliforms from 10^5 to 10^9 MPN/100 mL in waters of Indo-Gangetic riverine system used for irrigation of leafy greens. Irrigation water containing raw sewage or improperly treated effluents from sewage treatment plants may contain hepatitis A, Norwalk viruses, or enteroviruses in addition to bacterial pathogens (Beuchat, 1998). Regional differences in developed countries have also been observed (Kavka *et al.*, 2006).

Intraregional differences in microbial quality of surface waters are substantial. For example, in about 3500 surface-water samples from Ohio, 35% of the samples contained fewer than 126 colony-forming units (CFU) of *E. coli* per 100 mL, 13% contained between 126 and 235 CFU/100 mL, 20% contained between 235 and 576 CFU/100 mL, and 32% contained more than 576 CFU/100 mL (Stoeckel, 2009). A study of well water from 268 household and stock wells in an 1100 mi^2 area of southeast Nebraska showed that 37% of samples contained fecal coliforms at levels of up to 950 fecal coliforms per 100 ml of water (Exner and Spalding, 1985). In the absence of established relationships between indicator organisms and specific pathogens, it is impossible to evaluate the microbial contamination potential associated with any of these samples.

Manure applications have been often anecdotally implicated in creating differences in microbiological water quality of surface waters. Analysis of point source data in the survey of surface waters in southern Alberta, Canada showed that predicted manure output from cattle, pig, and poultry feeding operations was directly associated with prevalence of these pathogens (Johnson *et al.*, 2003). It was concluded that variations in time, amount, and frequency of application of manure to agricultural lands could have influenced levels of surface-water contamination. On the other hand, a survey in Iowa under auspices of the USDA Conservation Effects Assessment Project benchmark watersheds (Richardson *et al.*, 2008) found that *E. coli* populations can be large enough to impair the use of the waterways for contact recreation during much of the summer, but patterns do not always support the assumption that manure is the major source.

Microbial quality of well water can be affected by the design of wells, nature of the substrata, depth to groundwater and rainfall (Gerba, 2009). In the USA, the majority of drinking water disease outbreaks documented are caused by fecal contamination of wells (Reynolds *et al.*, 2008). Close *et al.* (2008) noted that there is greater filtration of pathogens in finer grained soils compared to coarser-stony soils, however, macropores may enable rapid transport of pathogens through otherwise fine-grained soils (e.g., Guber *et al.*, 2005). Deeper soils will generally filter out more pathogens than similarly structured shallow soils, minimizing groundwater contamination. The hydrological regime determines the amount of water available for leaching and transport of the pathogens. It impacts the travel time through the soil and vadose zone to the aquifer. The travel time is also affected by (a) the water content and structure of the soil and vadose zone materials; (b) geochemical properties and organic matter content of the soil and the properties of the microbial cells (affecting the adsorption and desorption of pathogens); and (c) the depth to groundwater table; deeper groundwater tables provide more time for pathogens to die off and/or be filtered before entering the groundwater system. Long distance transport of pathogens is possible in fractured limestone and clay soils, and gravel sandy soils (Gerba, 2009). A study by Johnson *et al.* (2010) found high occurrence of viral contamination (averaging ~50 MPN/100 L) in karst aquifers of East Tennessee, and further suggested that co-occurrence rates of viruses and bacterial indicators were higher for karst aquifers than for other aquifer types. Although size of viruses makes them better suited to travel in pore spaces, their interactions with surfaces of the solid matrix can make their transport comparable with the transport of bacteria and parasite oocysts. Unprotected wells routinely have lower microbial water quality than protected wells (e.g., Shortt *et al.*, 2003).

Overall, no explanatory model of interregional differences and intraregional variations in microbial quality of irrigation water has been

proposed to date. The minimum set of informative environmental parameters affecting the microbial water quality is lacking. This precludes large-scale estimates of microbial quality of irrigation waters based on environmental correlations.

2.2. Temporal and spatial variability

Pathogen and indicator organism concentrations in irrigation water sources may exhibit both diurnal and seasonal variability as well as be affected by precipitation events. No reports on diurnal variability of pathogen or indicator organism concentrations in irrigation waters have been found in the literature. However, the diurnal variability of *E. coli* concentrations was documented for surface water sources. The variation coefficient of $\log_{10}$ (concentration) of *E. coli* measured within a day in three Canadian streams by Meays *et al.* (2006) was about 0.2, and there was a clear trend to a decrease of concentration from morning to midday. Based on data of Whitman *et al.* (2004), the AM-PM differences between *E. coli* concentrations were about 0.5 log on sunny days and 0.2 log on cloudy days in Lake Michigan. On some of the sunny days, the decrease in *E. coli* concentration from AM to PM was up to 2 logs. Variability in replications for concentrations was substantially larger in the morning than that in the afternoon. The decrease of *E. coli* concentrations (MPN per 100 mL) in Massachusetts streams from 7 AM to 3 PM varied from 0.8 to 0.2 log (Traister and Anisfeld, 2006). The morning-to-afternoon decrease in concentrations of *Campylobacter* in rivers has also been observed (Eyles *et al.*, 2003; Obiri-Danso and Jones, 1999).

Rainfall events inevitably increase concentrations of pathogens and indicator organisms in streams, reservoirs, and ponds due to surface runoff into waterways and release of bacteria from bottom sediments (Pachepsky and Shelton, 2011). Alternatively, dramatic rainfall inputs can dilute surface waters and effectively decrease indicator concentrations. Working in a constructed wetland in Arizona, USA, McLain and Williams (2008) reported increased *E. coli* concentrations during months of no precipitation (avg. $\sim$400 CFU *E. coli*/100 mL^1), compared to samples collected during the summer monsoon ($<$100 CFU/100 mL^1) when 70 mm of rainfall was recorded. Seasonal variations in pathogen and indicator organisms were reported for various surface water sources. Patterns of seasonality were quite different in different regions. For example, observers in California usually reported higher concentrations during wetter months (e.g., Boehm *et al.*, 2002; Cooley *et al.*, 2007) and after heavy rainfall and related it to increased runoff. Levels of *Cryptosporidium* and *Giardia* in the Rio Grande water are much higher during the nonirrigation season (November through April), when the river flow is dominated by wastewater effluent, than during the irrigation season when releases from

Elephant Butte Reservoir and return flows increase the volume of river water, leading to as much as a 100-fold decrease in pathogen levels (DiGiovanni, 2004). Observers in the UK, on the other hand, indicated that recurring storm events deplete bacterial reservoirs in the watershed and therefore the lowest concentrations are found in the end of the sequence of rainfall events (e.g., Hunter *et al.*, 1992; Rodgers *et al.*, 2003). On the other hand, Eyles *et al.* (2003) attributed the highest Campylobacter concentrations in summer to the higher stocking rates and direct access of livestock to surface waters. Gannon *et al.* (2004) noted that most isolations of *E. coli* O157:H7 from surface water in southern Alberta occurred in summer. Research in the Eastern USA typically showed highest concentrations in the summer (Cinotto, 2005; Kim *et al.*, 2010; Shelton *et al.*, 2011; Traister and Anisfeld, 2006; Vereen *et al.*, 2007). Seasonality appears to be the consequence of the interplay of land use, water management, weather patterns, and specific organism properties and sources. Patterns other than seasonal or diurnal have also been discovered in stream flow concentrations of microorganisms (Koirala *et al.*, 2008).

Few studies exist reporting seasonal variations in microbiological quality of wastewater. Haramoto *et al.* (2006) examined seasonal quality of tertiary-treated effluent leaving a water treatment plant in Tokyo, Japan, and reported higher concentrations of human norovirus in winter, coinciding with the epidemic season in that country. However, Rock *et al.* (2009) examined reclaimed water leaving treatment plants in Arizona over a period of 1 year, and found no seasonal differences in indicator bacteria (*E. coli*, enterococci, *Salmonella*) or viruses.

Spatial distributions of pathogen and indicator organisms in surface water sources are usually highly asymmetrical with most sites having relatively low values, with a few sites having high values (e. g., Solo-Gabriele *et al.*, 2000; Tate, 2010). Presence of the hot spots in streams sometimes can be associated with point sources of pollution. Hot spots in reservoirs and impoundments could be related to aquatic plant and algal growth (Cinotto, 2005; Dewedar and Bahgat, 1995).

Overall, irrigation water from any surface source is likely to contain enteric pathogens at one time or another. Land use and climate affect site-specific concentrations of pathogens that can reach very high levels if raw wastewater is allowed to reach the surface water source. The concentrations and prevalence of pathogens in wastewater and contaminated surface water are much lower than that of indicator organisms. Spatial and temporal distributions of concentrations of pathogens are typically skewed, and datasets contain many relatively low values and few high values. Weather and land use patterns affect pathogen concentrations, but these relationships are difficult to establish due to high variability of pathogen concentrations.

3. Implications of Irrigation Water in Spread of Foodborne Diseases

Direct evidence of irrigation water causing foodborne disease is relatively rare. This is because a "cause-effect" relationship requires that (1) the same pathogenic strain be isolated from the patient, produce, and irrigation sources, and (2) there is a clear sequence of events connecting patient, produce, and irrigation source. In the absence of direct confirmation, the "cause-effect" relationship can only be inferred based on circumstantial or subjective evidence. Figure 1 illustrates the "cause-effect" relationships inferred or hypothesized in research of foodborne diseases caused by consumption of fruits and vegetables. These relationships have been summarized by Steele and Odumeru (2004) as:

- isolation of outbreak pathogens from produce and irrigation water (Fig. 1, links B and A), as well as the actual source (links C, B, and A);
- epidemiological investigations of food poisoning outbreaks implicating irrigated produce (links B and A), or contamination of produce via other vectors (links D and A); or
- observations of increased incidence of disease in areas practicing irrigation utilizing highly contaminated wastes (link E).

The inability often to identify the locations associated with produce contamination and delays inherent in foodborne outbreak field investigations reflect why the "cause-effect" relationships often are difficult to establish. Nevertheless, contaminated surface water in the vicinity of produce (Mandrell, 2011) and cross-contamination of irrigation water (U.S. FDA and California Food Emergency Response Team, 2008) have been suspected in some large produce outbreaks in the USA.

3.1. Epidemiological investigations of food poisoning outbreaks implicating irrigated produce

Despite the general belief that irrigation water poses a potential source of pathogens in food-borne outbreaks, there are relatively few confirmed cases in the USA. Greene *et al.* (2008) investigated a multistate outbreak of *Salmonella* Newport infection associated with eating tomatoes in the USA. Contaminated tomatoes were traced back to the eastern shore of Virginia, where the outbreak strain was isolated from pond water used to irrigate tomato fields. Two multistate outbreaks caused by one rare strain, and identification of that strain in irrigation ponds 2 years apart, suggested persistent contamination of tomato fields. Söderström *et al.* (2008) investigated the outbreak of verotoxin-producing *E. coli* in Sweden caused by the

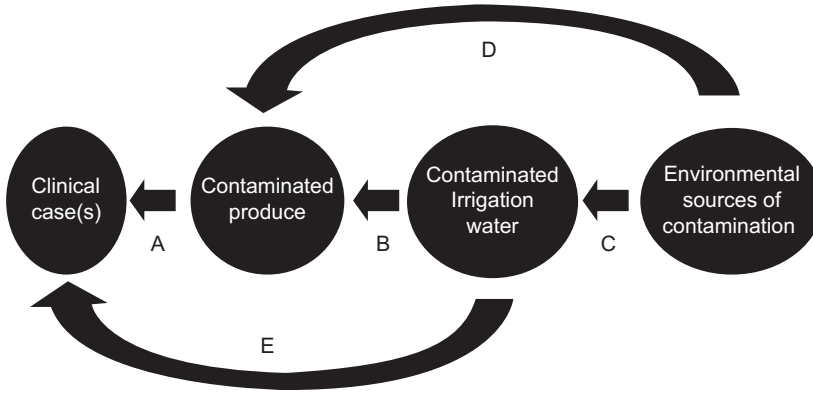

Figure 1 Inferences in research of irrigation water as a source of foodborne diseases caused by consumption of fruits and vegetables.

consumption of lettuce that was irrigated by water from a small stream. Identical verotoxin-producing *E. coli* O157 strains were isolated from the patients and in cattle at a farm upstream from the irrigation point. An *E. coli* O157:H7 outbreak in the USA associated with shredded lettuce (U.S. FDA and California Food Emergency Response Team, 2008), was traced back to the accidental mixing of well water, intended for irrigation, with water from a dairy manure lagoon. Finally, the same strain of *Salmonella* was found in irrigation water and in Serrano peppers implicated in an outbreak of Salmonellosis caused by a strain of *Salmonella saintpaul* (Centers for Disease Control and Prevention, CDC, 2008).

Additional studies are suggestive of a link between contaminated irrigation water and contaminated produce, although the evidence is circumstantial. In some instances, pathogens have been subsequently isolated from irrigation sources, although there was not a direct match with the outbreak strains. Duffy *et al.* (2005) isolated pathogenic bacteria from irrigation water, however, these were determined to be different from those isolated from cantaloupe because DNA fingerprinting was inconclusive. Cooley *et al.* (2007) reported results of the extensive sampling of a farm associated by traceback with three separate outbreaks in 2002–2003. The only sample yielding *E. coli* O157:H7 was a creek sediment sample collected adjacent to Farm A in July 2004. However, the strain was different from clinical strains associated with the three outbreaks. A matching outbreak strain was isolated from the river near where baby spinach was grown associated with a large 2006 outbreak (Cooley *et al.*, 2007; Mandrell, 2011). Although there was no direct evidence that the river water was related to the outbreak, an investigation team hydrologist suspected that the shallow aquifer supplying irrigation well water could have been recharged by this river water (Mandrell, 2011).

In other instances, forensic examination of foodborne outbreaks isolated no pathogens from irrigation water. For example, Ackers *et al.* (1998) reported on a case where irrigation water was implicated in outbreaks of *E. coli* O157:H7 infection from contaminated lettuce. The farm obtained its irrigation water from a nearby pond supplied by several streams that passed through cattle fields. Sampling of water and feces did not yield *E. coli* O157:H7. However, the environmental sources of potential water contamination were present, including improperly aged compost, feces of possibly infected cattle in the adjacent uphill pasture, cattle access to the streams above the pond used for irrigating the lettuce, and feces of other animal reservoirs of *E. coli* O157:H7, such as the sheep kept on the farm or deer. Hillborn *et al.* (1999) implicated irrigation water in an outbreak of *E. coli* O157:H7 attributed to mesclun lettuce, which was suspected to have been irrigated with water contaminated by dust from cattle grazing land. At the time of the environmental investigation, however, no *E. coli* O157:H7 was isolated from samples of well water, water from a cattle trough, water sampled from the cattle pasture, and cow or chicken manure. Irrigation water was also implicated in *C. cayetanensis* infections from raspberries (Herwaldt, 2000). In studies by Wachtel *et al.* (2002), irrigation water was implicated as a source of *E. coli* detected on cabbage seedlings irrigated with water inadvertently contaminated by a municipal sewage release; no *E. coli* were detected on seedlings in an adjacent field irrigated with municipal water. Although the source of the crop contamination could not be demonstrated conclusively because water samples tested negative for *E. coli*, the authors speculated that the creek water used for irrigation contained pathogenic bacteria associated with human waste or waste from wild animals. Rzeżutka *et al.* (2010) noted that *Cryptosporidium* sp. oocyst-contaminated vegetables originated from Polish districts with the highest numbers of homesteads possessing cattle herds, and no contaminated produce was detected from districts containing lower numbers of cattle-owning homesteads, strengthening the assumption that the origin of the contamination was livestock.

In contrast, no studies have established a relationship between irrigation water and disease outbreaks in the United Kingdom (Tyrrel *et al.*, 2006). Consequently, the potential for produce contamination from irrigation water has been established, but it is difficult to quantify the extent of the problem (Groves *et al.*, 2002).

Despite public perceptions that irrigation with reclaimed wastewater decreases microbiological food safety, no case of foodborne illness has been attributed to wastewater irrigation in the USA to date, except by unintended cross-contamination (U.S. FDA and California Food Emergency Response Team, 2008). Furthermore, risk assessments so far indicate that human health risks due to tertiary effluent irrigation is much lower than that deemed "acceptable" by public health standards (Zhao *et al.*, 2006).

3.2. Presence of pathogens in produce irrigated with contaminated water

Despite the limited number of confirmed or circumstantial cases of produce contamination from irrigation water, laboratory studies have elucidated potential mechanisms of produce contamination from water-borne pathogens. Laboratory and field studies show that pathogens and indicator organisms (e.g., generic *E. coli* and *E. coli* O157:H7) transmitted from irrigation water to produce can remain viable for variable periods of time depending on environmental conditions (e.g., Delaquis *et al.*, 2007). Nonpathogenic *E. coli* persisted for up to 28 days whereas *E. coli* O157:H7 did not survive for more than 14 days in inoculated spinach plants (Patel *et al.*, 2010). Pathogens survive and proliferate in sites where nutrients are available (Delaquis *et al.*, 2007; Kroupitski *et al.*, 2009a) and consequently, the plant rhizosphere has been shown to be a reservoir for opportunistic human pathogenic bacteria (Berg *et al.*, 2005). It is suggested that survival of human pathogens is augmented by inclusion in plant phyllosphere biofilms or internalization within the plant (Heaton and Jones, 2008). Similar to plant-associated bacteria, pathogenic bacteria use cellulose and aggregative fimbriae for their attachment to plant surfaces (Mandrell *et al.*, 2006; Teplitski *et al.*, 2009). Lapidot and Yaron (2009) observed that the transfer of *Salmonella* to parsley leaves via irrigation water was dependent on curli forming abilities of the strains. In a recent study, Patel *et al.* (2011b) reported significantly higher attachment of curli-expressing *E. coli* O157:H7 on iceberg lettuce and cabbage than the attachment of curli-negative *E. coli* O157:H7 strains.

3.2.1. Pathogen pathways into plants

Pathogens can enter vegetable plants and become internalized, that is, colonize some plant tissues. Early studies suggested that *E. coli* could be transported into the edible part of lettuce from soil through root system (Solomon *et al.*, 2002b), or that *Salmonella* Newport could be transported from contaminated roots to the aerial parts of Romaine lettuce seedlings depending on the developmental stage of the plant (Bernstein *et al.*, 2007b). However, more recent studies have not confirmed these results. *E. coli* was found in root tissue but not in shoot tissue of spinach plants grown on inoculated soil (Sharma *et al.*, 2009). Jablasone *et al.* (2004), Miles *et al.* (2009), Zhang *et al.* (2009), Erickson *et al.* (2010) found that internalization of *E. coli* and *Salmonella* via the root system does not occur or is an extremely rare event.

Pathogens may enter aerial portion of plants through stoma, scar tissue, or wounds as a consequence of irrigation water contacting leaf surfaces or from raindrop splashes from the soil surface (Kroupitski *et al.*, 2009b; Materon *et al.*, 2007; Mitra *et al.*, 2009). Guo *et al.* (2001) observed migration of *Salmonella* from soil directly into the stem scar tissue of green tomatoes.

Wound surfaces seemed to be suitable for *E. coli* to enter iceberg lettuce tissues (Barker-Reid *et al.*, 2009) and promoted its survival (Aruscavage *et al.*, 2008; Brandl *et al.*, 2004). Stomatal cavity was the preferential port of entry of *E. coli* internalization to the vegetable leaf in experiments of Gomes *et al.* (2009) with four different varieties of lettuce. A possible pathway from soil to stomata along the wet stem surface has not been explored to date.

3.2.2. Adherence to plants

Any pathogen may reach plant surfaces via irrigation water; however the potential for adherence is both strain and plant specific. For example, strain-specific properties of *Salmonella* (curli and cellulose) affected its ability to enter parsley plants from contaminated irrigation water in the work of Lapidot and Yaron (2009). Substantial differences in survival on and in tomato plants were observed for various serotypes of *Salmonella* by Guo *et al.* (2001).

Plants also differ in their propensity to become contaminated with pathogens when irrigated with contaminated water. Quantitative risk assessment models for the use of reclaimed water show that risk varies between crops, with lettuce found to pose a higher risk than cucumber, but comparable to that of broccoli and cabbage (Hamilton *et al.*, 2006). The prevalence of total *E. coli* was significantly higher in both organic and conventional lettuce than in any other produce varieties in the study of Mukherjee *et al.* (2004). When *E. coli* and *Clostridium perfringens* were added to irrigation water that was supplied in furrows and in drippers, microorganisms were detected on the surfaces of cantaloupe and lettuce, but were never recovered on the bell peppers (Song *et al.*, 2006). Irrigation with contaminated water resulted in concentrations of total coliforms in amaranthus much higher than in other vegetables in the study of Okafo *et al.* (2003). Those crops whose edible parts develop on the ground surface, such as lettuce and parsley, were more contaminated with *Salmonella* than those that grow above the soil surface, like tomatoes and pimento in the work of Melloul *et al.* (2001). The USDA-AMS Microbial Data Program also shows high levels of *E. coli* on these items (USDA-AMS-MDP, 2009).

There are indications that differences between cultivars may influence the extent of contamination from irrigation water to different levels (e.g., Barak *et al.*, 2008 for tomatoes, and Mitra *et al.*, 2009 for spinach), although reasons for that are currently unknown.

3.2.3. Effect of the concentration

The evidence for whether the initial concentration of pathogens in irrigation water is critical for produce contamination is mixed. For example, work of Webb *et al.* (2008) shows a positive relationship between the *E. coli* O157:H7 concentrations and incidence on spinach. Whereas no *E. coli* O157:H7 was detected on spinach plants spray-irrigated with water

with 10^2 *E. coli* O157:H7 CFU ml^{-1} in that study, the pathogen was found at higher concentrations of 10^4 CFU ml^{-1} and the incidence increased when the concentrations were increased to 10^6 CFU ml^{-1}. *Salmonella* were undetectable by MPN analysis on spinach plants during a 6-week study with plants irrigated repeatedly with water containing 10^3 CFU ml^{-1} (Patel and Darlington, 2010). *Salmonella* persisted at a levels of 10^4 CFU $plant^{-1}$ after 24 h after plants were irrigated with very high levels of the pathogen (10^6 CFU ml^{-1}). These results imply lower concentrations correspond to lower incidence of contamination. On the other hand, Mootian *et al.* (2009) observed that lettuce irrigated with water containing *E. coli* O157:H7 in amounts as low as 10^1 or 10^2 CFU ml^{-1} may become contaminated. They reported that 30% of the mature plants initially irrigated with contaminated water for 15 days were positive for *E. coli* O157:H7. The concentration in irrigation water may not necessarily be the dominant factor if the microorganism is able to internalize in produce or colonize it without being outcompeted by the plant internal microbial community of the plant phyllosphere. However, there is no direct evidence that this route of contamination is a significant factor in contamination of any produce.

3.3. Increased incidence of disease in areas practicing irrigation with high concentrations of pathogens in water

As long as 60 years ago, Norman and Kabler (1953) observed that poor microbiological quality of irrigation water was associated with the incidence of human pathogens in leafy vegetables. Connections between contaminated irrigation water and clinical studies (Link E in Fig. 1) are typically reported in areas where irrigation water may have unsatisfactory microbial quality, most often having waste origin. Katzenelson *et al.* (1976) compared the incidence of enteric communicable diseases in 77 kibbutz settlements practicing wastewater spray irrigation with partially treated nondisinfected oxidation pond effluent with disease in 130 kibbutz settlements practicing no form of wastewater irrigation. The incidence of shigellosis, salmonellosis, typhoid fever, and infectious hepatitis was two to four times higher in communities practicing wastewater irrigation during the irrigation season, whereas no differences were found for enteric disease rates during the winter nonirrigation season. A study in Mexico compared incidence of diarrheal disease and microbial quality of the irrigation water in 2320 households irrigating vegetables with either untreated wastewater or natural rainfall (Cifuentes, 1998). Rates of diarrhea were significantly higher in households irrigating with untreated wastewater than in households irrigating with rainfall alone. In Morocco, crop irrigation with untreated wastewater caused a significantly higher rate of salmonellosis in children of agricultural workers (39%) than in the children of nonagriculturalists (25%) (Ait Melloul and Hassani, 1999).

Populations near microbiologically contaminated surface water sources can be affected via transmission pathways other than irrigation such as aerosols from the surface microlayer as demonstrated in marine environments (Aller *et al.*, 2005), or transfer from domestic animals, insects, etc. Animal and composting facilities are preeminent sources of airborne particulates and dust as well as insects that vector enteric pathogens, however, emission rates, transport, survival, and deposition of particulates and insects carrying *E. coli*, *Salmonella*, and other fecal bacteria from these sources currently are not quantified (Duan *et al.*, 2008; Millner, 2009). Insect vectors may harbor and subsequently transmit *Enterobacteriaceae* and plant pathogens to plants and animals by direct physical contact and their frass (Mitchell and Hanks, 2009). Information about such transport is very limited, but potential vectors for contamination of leafy greens have been identified and studied (Talley *et al.*, 2009).

Creation of irrigation water storages affects local ecological systems, and can modify pathogen transmission. Ecosystem changes concomitant with irrigation development in Sri Lanka, for example, resulted in long-term changes in the composition of the mosquito fauna, which was characterized by the increasing dominance of species with the potential to transmit human pathogens (Amerasinghe and Indrajith, 1994). Low microbial quality of water can be translated in higher disease incidence not only via agricultural production but also via household uses, including drinking unboiled water (Cifuentes, 1998; Van der Hoek *et al.*, 2001).

In summary, transmission of pathogens to produce and their subsequent survival are evident by incidence studies and multiple recent outbreaks described above and in reviews (Mandrell, 2011). However, details of the potential mechanisms of transport have been documented mostly in laboratory studies. More field data are needed to establish reservoirs and patterns of transmission occurring in farm operation environments, and to evaluate the relative importance of various factors such as pathogen concentration, pathogen strain, plant state, irrigation regime, weather patterns, etc. Results of studies of the incidence of *E. coli* O157:H7 and *Salmonella* in watersheds and other environments in a major produce production environment of California emphasize the need for more specific data about these factors (Cooley *et al.*, 2007; Gorski *et al.*, 2011).

4. Standards, Guidelines, and Risk Assessment

With increased recognition of the importance of microbiological quality of irrigation water and its impact on food safety and public health, the need for regulation has become obvious. Guidelines and standards are the two means used to regulate food safety. For the purpose of this review,

standards are defined as regulatory documents containing specific numerical limits on concentrations of some microorganisms, whereas guidelines are issuances that do not contain specific concentration limits. Standards may contain elements of guidance. This review is not concerned with the often used distinction that standards are enshrined in law whereas the guidelines are not covered by law (Agriculture and Agri-Food Canada, 2007).

4.1. Current standards for microbial quality of irrigation water

Microbiological water quality standards are based on indicator organisms that, albeit not pathogenic, are presumed to correlate with pathogens, thus facilitating estimation of the probability that potential pathogens are present. A large number of microorganisms have been proposed and tested as indicators (Ashbolt *et al.*, 2001), although only a small number of them have been adopted in standards (Table 1). The earliest standards used "total coliforms" as the indicator organism (U. S. EPA., 1973). However, because fecal contamination was considered the to be the probable source of pathogens in waters, microorganisms in feces were selected as more appropriate indicators. Subsequently, standards were based on the thermotolerant "fecal coliforms", a subset of total coliforms that have the ability to grow and ferment lactose and produce acid and gas at 44.5 °C. Most recently, the indicators of choice have become *E. coli* and, in some cases, fecal streptococci (Table 1). Additional standards have been adopted to include also nematode and/or helminth egg counts (e. g., Blumenthal *et al.*, 2000).

Microbiological water quality standards for irrigation water should include distinctions between irrigation water sources, method of irrigation, type of crop, and land use (Table 1). For wastewater, an important distinction was introduced between restricted irrigation (that is, for uses that include crops likely to be eaten uncooked) and unrestricted irrigation for crops that will be cooked (Blumenthal *et al.*, 2000 and Marr, 2001). Some states do not allow irrigation of food crops with wastewater effluents of any quality. For example, Florida does not allow spray irrigation with effluent water of edible crops that will not be peeled skinned, cooked, or thermally processed before consumption (U.S. EPA, 2004), though drip and subsurface irrigation are allowed (O'Connor *et al.*, 2008). However, it has been noted that the requirements for treated wastewater are more restrictive, in some cases, than drinking water standards (O'Connor *et al.*, 2008). The National Research Council (NRC) (1996) reported that the quality of treated effluent for most parameters is generally well below the levels measured in the Colorado River and the recommended minimum irrigation water quality criteria. Higher concentrations of indicators are tolerated in surface irrigation when irrigation water does not come in contact with edible parts of plants.

Table 1 Examples of microbiological water quality standards applied to waters used to irrigate produce

Source	Type of water	Irrigation method	Land use	Type of crop	Concentration limits				
					TC (total coliforms, cell/100 mL)	FC (fecal coliforms, cell/100 mL)	EC (*E. coli*, cell/100 mL)	FS (enterococci, cell/100 mL)	NE (nematode eggs/L)
U. S. EPA. (1973)	Surface	ns	ns	ns	ns	1000[a]	ns	ns	ns
Canadian Council (1999)	ns	ns	ns	ns	1000[a]	100[a]	ns	ns	ns
Alberta Environment (1999)	Surface	ns	ns	ns	1000[b] 1000[c] 2400[a]	100[b] 200[a]	ns	ns	ns
Warrington (1988)[d]	ns	ns	ns	Eaten raw	1000[c] 2400[a]	200[b] 200[a]	77[b]	20[b]	ns
Warrington (1988)	ns	ns	Open to public and grazing	Other then eaten raw	1000[c] 2400[a]	ns	385[a]	100[a]	ns
Williamson (2002)	ns	ns	ns	ns	ns	200[a]	200[a]	ns	ns
Anonymous (2006)	Surface	ns	ns	Eaten raw	1000[a]	100[a]	ns	ns	ns
Blumenthal *et al.* (2000)	Wastewater	ns	ns	Eaten raw	ns	1000[a]	ns	ns	1[a]
Blumenthal *et al.* (2000)	Wastewater	ns	ns	Eaten processed	ns	100000[a]	ns	ns	ns

CSFSGLLGSC (2009)[f]	ns	Over-head	ns	Eaten raw	ns	ns	126[b] 235[a]	ns	ns
CSFSGLLGSC (2009)[f]	ns	Drip/ furrow	ns	Eaten raw	ns	ns	126[b] 576[a]	ns	ns
Vermont Water Agency (2009)	ns	ns	ns	ns	ns	200[a]	77[a]	ns	ns
Johnson (2009)	ns	Over-head	ns	ns	ns	200[a]	126[a]	ns	ns
Johnson (2009)	ns	Drip	ns	ns	ns	576[a]	ns	ns	ns
Bahri and Brissaud (2004)	Wastewater	Over-head, surface	ns	Vegetables	1000[e]	1000[e]	ns	ns	ns

ns, not specified.
[a] Any single measurement.
[b] Moving geometric mean from five weekly measurements.
[c] Any from the five consecutive weekly measurements.
[d] *Pseudomonas aeruginosa* also limited.
[e] In at least 80% of consecutive measurements.
[f] Commodity Specific Food Safety Guidelines for the Lettuce and Leafy Greens Supply Chain.

Of growing concern in the use of wastewater for irrigation is the potential for these waters to contain organic contaminants (e.g., antibiotics, endocrine disrupting compounds, pesticide residues). Concentrations of these emerging contaminants are as yet unregulated and their effects on public and environmental health are poorly understood (Metcalf and Eddy, 2007).

The regional differences between water quality standards can be large. An example can be found in Table 1 where standards are shown for four Canadian provinces: Alberta Environment, Environmental Service, Environmental Sciences Division and Natural Resources Service, Water Management Division (1999), British Columbia (Warrington, 1988), Manitoba (Williamson, 2002), and Saskatchewan (Anonymous, 2006). Another example can be found in Table 2 where the wastewater microbiological quality standards are collected for the U.S. states that have such standards, and allow treated waste water to be used to irrigate fresh produce. Note that some state standards contain the maximum allowed concentration in any single sample, whereas, other states do not include this value. Marked differences in concentrations are approved also for surface versus overhead versus drip irrigation, possibly related to a finding that populations exposed

Table 2 Reclaimed wastewater standards for irrigation of raw eaten crops in U.S. states where such irrigation allowed

State	Irrigation method	Total (TC) or fecal (FC) coliforms	Median	Any single sample	Sampling frequency
Arizona	Overhead	FC	0[a]	23	Daily
Arizona	Surface	FC	200[a]	800	Daily
California	Overhead	TC	2.2[a]	23	Daily
Colorado	Overhead	TC	2.2[b]	NS	Daily
Hawaii	Surface only	FC	2.2[b]	23	Daily
Idaho[$]	NS	TC	2.2[b]	NS	Daily
New Jersey	Surface only	FC	2.2[a]	14	Daily
New Mexico	Surface only	FC	1000	NS	NS
Oregon	Surface only	TC	2.2[b]	23	Daily
Texas	Surface only	FC	20[c]	75	Twice a week
Utah	Overhead	FC	0[a]	14	Daily
Washington	Surface only	TC	2.2[b]	23	Daily

Numbers are concentrations of total or fecal coliforms, cells/100 mL (U. S. EPA, 2004).
NS, not specified.
[a] Detected in 4 of last 7 daily samples.
[b] 7-Day median.
[c] Median from twice-a-week samples.

to aerosols from sprinkler irrigation may have increased risks of enteric viral and bacterial infections (Blumenthal and Peasey, 2002). However, it must be noted that increased risk was apparent only from irrigation water containing $>10^5$ TC/100 mL, orders of magnitude higher than permitted levels (Table 2). It is not clear what is the scientific or epidemiological rationale behind any specific regional standard.

Because of the scarcity of information of how microbiological water quality affects pathogen concentrations in produce and therefore consumer health, some regional irrigation water quality standards have been based on microbiological standards for recreational water (e.g., Söderström *et al.*, 2008 in Sweden, or CSFSGLLGSC, 2009, in California). The use of recreational water standards is considered to be problematic because they were established assuming human health risk posed by full-body contact during swimming, and therefore do not take into account the rapid die-off during post-irrigation intervals and exposure to environmental stresses associated with crop production (Suslow, 2010). Given that abstention of overhead irrigation is viewed as an important management practice to increase microbial safety of produce (e.g., Barker-Reid *et al.*, 2009), the irrigation regime before harvest should be factored in the standards. For example, the California Leafy Greens Marketing Agreement (LGMA) requires a 24-h wait period between irrigation and harvest.

Depending on the region, distinctions in standards have been made between irrigation water sources, irrigation method, type of the crop, and land use (Table 1). The permitted concentrations of indicator organisms are usually much higher in case of surface irrigation method where water does not come in contact with edible parts of plants. This assumption requires further scrutiny because it has recently been suggested that (1) pathogens can enter plants via root systems (Bernstein *et al.*, 2007a,b; Solomon *et al.*, 2002b), and (2) the in-field splash can transport microorganisms from the soil surface quite far (Boyer, 2008).

Microbiological standards have been scrutinized and criticized. The two major criticisms are (a) water sampling frequency has never been justified, and (b) indicator organism concentrations do not correlate with concentrations of pathogens. Recommendations on sampling frequency fluctuate widely from: annual sampling (Anonymous, 2010b), and sampling not more than 1 month apart (Gombas, 2007; Strang, 2010), five times a month (Jamieson *et al.*, 2002), to daily (e.g., Table 2). A fairly common requirement is to use the geometric mean from five weekly measurements (Table 1). The problem arises from high temporal variabilities in indicator organism concentrations that have been observed in irrigation water sources (see Section 2 of this review). The variabilities and ranges of indicator concentrations from the geometric means and maximum of four once-a-week measurements have not been reported, nor their relevance to produce contamination was established.

Data on correlations between concentrations of indicator organisms and pathogenic or potentially pathogenic organisms are inconclusive at best. Several studies attempting to find such correlation have found none (Duris *et al.*, 2009; Harwood *et al.*, 2005; Jjemba *et al.*, 2010; Kramer *et al.*, 1996; Shelton *et al.*, 2011). Differences in indicators due to environmental fitness for survival, or even their ability to multiply in the environment, all influence indicator usefulness (Ashbolt *et al.*, 2001). Hence, viral, bacterial, parasitic protozoan and helminth pathogens are unlikely to all behave in the same way as a single indicator group, and certainly not in all situations. It is worth noting that some drinking water outbreaks occurred from water where coliform standards had been met (Craun *et al.*, 1997; Marshall *et al.*, 1997).

Standards for drinking and recreation waters have their origin in epidemiological studies. No such studies have been carried out for irrigation waters. Produce is transported in large lots and dissemination of pathogens from a few contaminated plants to others is possible. This, and the reported independence of pathogen internalization in plants on concentration in irrigation water (see Section 3 of this review), challenges the application of concentration-based standards.

Concentrations of indicator organisms in water do not reflect the ecology of pathogens and indicators in water sources. Some higher aquatic organisms and soil organisms can harbor both pathogen and indicator microorganisms (Barker *et al.*, 1999; Bichai *et al.*, 2008). Many members of the total coliform group and some so-called fecal coliforms (e.g., species of *Klebsiella* and *Enterobacter*) are not specific to feces, and even *E. coli* has been shown to grow in natural aquatic environments (Pachepsky and Shelton, 2011). Hence, whereas indicators representing fecal contamination in temperate waters are *E. coli* and enterococci, *E. coli* and enterococci may grow in tropical water and soils (Pachepsky and Shelton, 2011) and alternative indicators should be considered. Use of multiple indicators was suggested based on a better understanding of the types, occurrence and concentration of pathogens (Gerba and Rose, 2003).

The difficulty of associating specific indicator concentrations with fresh produce–related health risks does not negate the value of measuring these concentrations. High indicator organism concentrations indicate high levels of fecal contamination and, therefore, an elevated probability that fecal pathogens could be present. However, the use of these measurements is currently a contentious issue aggravated by an extremely low knowledge base regarding health issues related to the quality of irrigation water.

4.2. Role of microbiological water quality standards

There are three schools of thinking on the usability of pathogen and indicator concentration-based standards for irrigation water quality: (a) setting indicator-based standards and adhering to them as mandated by

regulatory agency or marketing body, (b) using standards as the auxiliary tool in the guidance to control microbial contamination of produce, and (c) not using standards at all.

Setting standards may be a convenient regulatory measure. However, it may be difficult to implement. Tyrrel (1999) noted that in the UK "one obvious practical problem is that there are many more irrigation abstraction points than potable water abstraction points in the river network which could make compliance with any proposed regulations very difficult to manage." The irrigation water monitoring infrastructure is absent and no research has been to done to evaluate the feasibility of such infrastructure.

Complementary use of standards is advocated in many regional or local guidelines. The Code of Practice for Food Safety in the Fresh Produce Supply Chain in Ireland, for example, states that "testing of agricultural water for micro-organisms and chemicals, whilst important should not be used as the sole method of controlling water-borne hazards. Water testing results can vary considerably and only reflect the water quality at the time of sampling. Growers should focus on the adoption of good agricultural practices to control water-borne hazards and use water testing as a means of validating these practices" (Food Safety Authority of Ireland, 2001). However, which standards to use in such case is an open question. Purely subjective selections have been recommended such as "Water quality standards published by the U.S. Environmental Protection Agency for either swimming, shellfish growing waters, or drinking water may serve as a guideline, depending on how strict public health officials or growers, shippers, and retailers of leafy green vegetables want to be" (Extension Foundation, 2010).

U. S. FDA (2009) put forward the "Draft Commodity Specific Guidance Documents for Leafy Greens, Melons, and Tomatoes" without any reference to microbiological water quality standards. In the absence of scientific data on efficient testing of irrigation water, the regulatory agency provided the list of essential points to look at, questions to ask, and practices to apply to reduce the risk of microbial contamination of leafy greens. A similar situation was acknowledged in using wastewater for irrigation. "The information on biological quality of the water or associated epidemiology is not understood well enough to develop standards. There is no evidence that regulations or standards are needed, but there is sufficient information to indicate that considerable caution and adherence to recommended practices are essential to protect human health" (McFarland *et al.*, 2007).

Instead of "using one size fits all" standards, the U. S. FDA guidance emphasizes site-specific analysis for specific crop, pathogen, irrigation system, water sources, and management. One methodology for such analysis—quantitative microbial risk assessment, or QMRA—has been first applied to wastewater irrigation and currently is actively applied to irrigation with water from other sources.

4.3. Quantitative microbial risk assessment

Risk assessment, in general, is the characterization and estimation of potential adverse health effects associated with exposure of individuals or populations to hazardous materials or situations. With regard to irrigated produce, adverse health effects may be caused by the ingestion of pathogens with the produce, by inhaling aerosols containing pathogens, by the unintended consumption of contaminated water, etc. The quantitative microbial risk assessment for irrigation waters, or irrigation QMRA, establishes a relationship between the concentrations of pathogenic microorganisms in irrigation water and the probability of illness. Comprehensive introductions in QMRA in general and in irrigation QMRA have been published (Haas *et al.*, 1999; Petterson and Ashbolt, 2003).

QMRA employs two statistical models: the exposure model and the infectivity model. There is no single mathematical formulation for the irrigation QMRA. An outline of one simple implementation of the irrigation QMRA is shown in Fig. 2. The exposure model in Fig. 2 computes the daily dose as the product of concentration of the pathogen in irrigation water, volume of irrigation water interacting with the unit mass of produce,

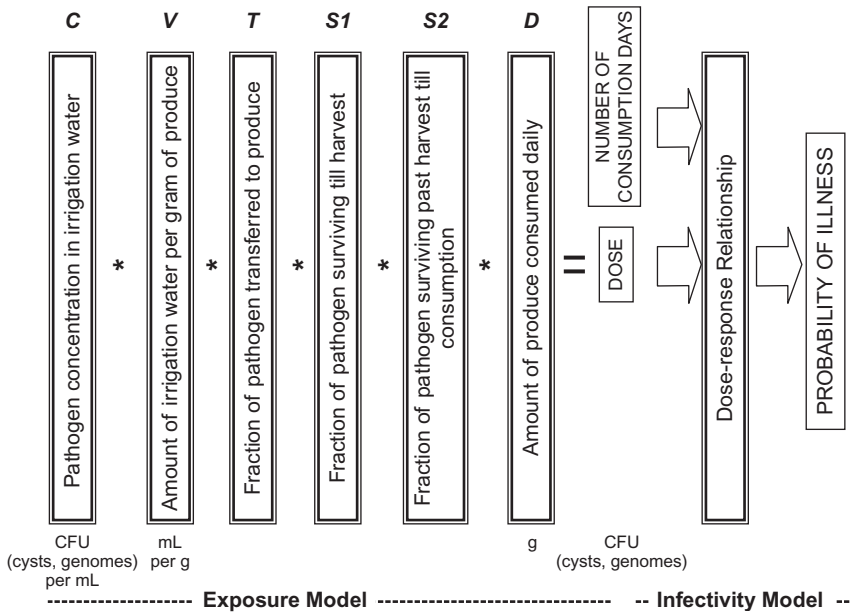

Figure 2 The outline of the quantitative microbial risk assessment model to assess the risk of illness due to consumption of produce irrigated with pathogen-contaminated water (modified from Stine *et al.*, 2005).

fraction of pathogen in produce that remained infective at harvest time, fraction of pathogen that remained infective between harvest and consumption, and the mass of produce consumed daily. The infectivity model uses the dose and the number of consumption days as inputs and provides the probability of illness as the output. The irrigation QMRA can be developed for specific pathogens, the agricultural management, the water source, the consumer group, and the environmental conditions.

The irrigation QMRA is rapidly evolving. It was first developed for the wastewater irrigation, and was adopted by the World Health Organization (WHO) in the development of guidelines for water-related diseases (Fewtrell and Bartram, 2001). Currently, QMRA is applied to other sources of irrigation water as well. Earlier versions of QMRA supported the use of indicator organisms and employed conversion of indicator organism concentrations to concentrations of the pathogen of interest (Blumenthal *et al.*, 2000). Later pathogen-specific irrigation QMRA were developed, notably for viruses on lettuce (Petterson *et al.*, 2001), enteric virus infection on cucumber, broccoli, cabbage, or lettuce (Hamilton *et al.*, 2006), *Cryptosporidium* and *Giardia* on irrigated tomatoes, bell peppers, cucumbers, and lettuce (Mota *et al.*, 2009), and norovirus and Ascaris infection (Mara, 2010).

Original QMRA formulations for vegetables irrigated with wastewater (Shuval *et al.*, 1997) treated inputs of the exposure model (*C*, *V*, *T*, *S*1, *S*2, and *D* in Fig. 2) as constant values. Later it was argued (Tanaka *et al.*, 1998) that these variables are actually uncertain and using multiple results of QMRA, each computed with randomly selected values of the exposure model inputs, would produce a more realistic risk assessment in which the uncertainty of the probability of illness could be established. For that purpose, computer programs were developed to perform the Monte-Carlo simulations of QMRA (Mara and Sleigh, 2010).

One of the developments in irrigation QMRA applications has been its use to establish irrigation water standards. This application is based on using QMRA in reverse. Inspection of Fig. 2 shows that QMRA with constant *C*, *V*, *T*, *S*1, *S*2, and D can be used both as the forward procedure and in reverse. The information flow in Fig. 2 is from left to right for the forward procedure and from right to left for the reverse. The reverse procedure uses the given probability of illness as the input value and computes the dose by inverting the dose-response model. Then, knowing dose and *V*, *T*, *S*1, and *S*2, one can compute *C*, that is, find the pathogen concentration in irrigation water that causes the given probability of illness. This method of irrigation water standards development was suggested by Stine *et al.* (2005) who determined the concentrations of hepatitis A virus (HAV) and *Salmonella* in irrigation water necessary to achieve a 1:10,000 annual risk of infection. The inputs used in the exposure model were those that provided the worst case scenario. So far, we are not aware of irrigation water standards

that have been developed with reverse QMRA using exposure inputs as random uncertain values.

The advantage of QMRA is the potential to tailor it for the case at hand. Irrigation scheduling before harvest, preharvest environmental conditions, and type of crop should be considered in microbial water quality standards for irrigation water (Stine *et al.*, 2005). Where the experimental data were present, irrigation QMRA was developed for specific varieties of vegetables (Hamilton *et al.*, 2006), for specific age groups (Mara and Sleigh, 2009), and for different irrigation methods (Stine *et al.*, 2005), etc. The strength of QMRA is in its provision to assess treatment step performance needs and identify zones for critical control (Petterson and Ashbolt, 2003).

The irrigation QMRA obviously has many avenues for improvements. Literature, including some materials of the Section 2 of this review, shows that the survival of pathogens in produce is a complex process, not necessarily following a simple exponential decay pattern. Long term persistence of pathogens in produce may undermine the current focus exclusively on the die-off period between the last irrigation and harvest. Another important limitation of existing irrigation QMRA is that currently they are partially or completely relying on laboratory measurements. Field-scale observations and measurement of the exposure model parameters are scarce, and the need for them is acute. Other improvements in QMRA models include modifications in the structure and components of both exposure and infectivity models. This should lead to realization of potential applications of the irrigation QMRA framework in helping to establish guidelines and standards regarding microbial quality of irrigation water.

5. Fate and Transport of Pathogenic and Indicator Microorganisms in Irrigation Systems

Information on the fate and transport of pathogenic and indicator organisms is actively used in water quality-related regulatory, engineering and research fields. This information is needed by regulators to inform Total Maximum Daily Load estimates in the USA and Programmes of Measures in Europe, both of which are designed to prevent impairment of water quality at locations where compliance is assessed against health-based standards for drinking, bathing, or shellfish harvesting (Kay *et al.*, 2007).

Information on fate and transport of pathogenic and indicator organisms is also highly relevant at locations where the microbial quality of irrigation water is of interest. The general layout of fate and transport issues pertinent to irrigation water quality is shown in Fig. 3. The microbial quality of irrigation water is impacted by diverse agricultural, wildlife, and human

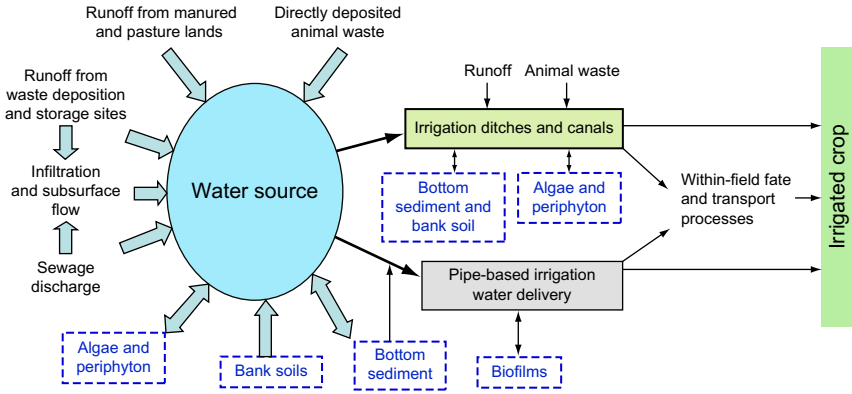

Figure 3 The layout of the processes affecting the microbial quality of the irrigation waters.

inputs including: runoff from land-applied manure and pasture lands, direct fecal deposition from cattle, overflow from manure lagoons and storage sites, fecal deposition from wildlife, discharge from leaky sewer lines, and subsurface flow from septic drainage fields. In addition, microbial concentrations in the water are dependent on exchange with microbial reservoirs in direct contact with water, including bottom sediment, periphyton, algae, and bank soils.

The microbial quality of irrigation water is also impacted by processes that occur during storage and in the distribution system. Changes can occur during transportation from the source to the field. Irrigation water transport via irrigation ditches and canals involves interaction with microbial reservoirs of bottom sediment, bank soils, algae and periphyton, whereas water transport via pipes involves interactions with biofilms in the pipes. Degradation in microbial water quality within transport pipelines has been shown to be especially pronounced in reclaimed water distribution systems. Jjemba *et al.* (2010) reported regrowth of multiple opportunistic pathogens, including *Legionella* and *Aeromonas*, in treated effluent systems leaving four U.S. municipal wastewater plants. Such regrowth has been associated with the presence of high levels of assimilable organic carbon, which serves as an energy source for bacterial growth, in reclaimed water (Ryu *et al.*, 2005; Weinrich *et al.*, 2010). Irrigation water collection, replenishment, and delivery occur in complex ecological systems that affect the microbial quality of water in many ways.

Substantial reviews have been published describing both general patterns of microorganism fate and transport in watersheds (Ferguson *et al.*, 2003; Kay *et al.*, 2007), and on specific pathogenic and indicator microorganisms, for example, *Salmonella* (Gorski *et al.*, 2011; Haley *et al.*, 2009; Walters *et al.*,

2011), *E. coli* O157:H7 (Cooley *et al.*, 2007) and *Cryptosporidium* and *Giardia* (Mohammed and Wade, 2009). One important conclusion is that the current state of knowledge does not allow for accurate predictions of the survival patterns for specific pathogens in specific irrigation water or microbial reservoirs, even though factors of pathogen survival and patterns of pathogen microbial population changes in time are generally known. The heterogeneity of the natural environments is very high and fecal indicator and enteric pathogen contamination are dynamic; it will be complicated to assess the appropriate measurements for natural waters and microorganism characterization (e.g., genotype, fitness) to predict die-off and/or growth of any specific pathogen (van Elsas *et al.*, 2010).

The absence of accurate predictions does not preclude using common sense for development of management actions that would aim to keep concentrations of pathogens below epidemiologically acceptable levels. Development of such actions has to rely on the information on fate and transport of microbial pathogens in environmental media present in irrigation systems. Knowing patterns of pathogen survival provides possible ranges of pathogen survival rates and allows quantification of the uncertainty of predictions. Currently there is no single source where existing data on pathogen fate has been collected and can be retrieved. The first database of this type has been developed only recently (Pachepsky *et al.*, 2009). Sources other than water must be considered because of known roles as microbial reservoirs.

5.1. Survival of pathogen and indicator organisms in waters suitable for irrigation

The majority of available data on pathogen and indicator survival has been obtained in water sources potentially suitable for irrigation. Consequently, it must be assumed that the processes occurring in various surface bodies of water are generally applicable to waters intended for irrigation. It must be noted that microbial die-off rates, detailed below, assume no additional inputs of water contamination following the initial measurements of viable populations. However, it has been shown that water quality often degrades quickly in storage ponds, due to inputs from avian or other wildlife (Higgins *et al.*, 2009; McLain and Williams, 2008).

Experiments on survival of pathogen and indicator organisms in natural waters usually demonstrate a decrease in microbial concentrations with time. However, the concentration decrease does not necessarily begin with the initial inoculation or input. An initial phase of growth, or lag phase, has been noted in experiments with *E. coli* and *Salmonella* in warm estuarine environments (Chandran and Hatha, 2005; Rhodes and Kator, 1988), in experiments with fecal streptococci and *E. coli* in tropical fresh waters (Wright, 1989), and *E. coli* in Southern Ontario (Dutka and Kwan,

1980). The duration of the observed growth and lag phase was from 1 to 3 days after which the microbial concentrations decreased.

The decrease in concentrations after or in absence of initial delay is typically exponential. At some point in time, the rate of the exponential decay may change, in which case, a biphasic decay is said to occur (Fig. 4). The review by Hellweger *et al.* (2009) shows that biphasic decay is a common phenomenon for *E. coli*, as well as for other microorganisms.

The rate of decay during the first inactivation phase k_1 is defined as the slope of the regression 'time versus natural logarithm of concentration' between $T1$ and $T2$ in Fig. 4. The reported values of k_1 for indicator and pathogen organisms vary by up to two orders of magnitude (Fig. 5). Based on experiments with *Salmonella* in Poland (Budzińska *et al.*, 2009) and with *E. coli* in Finland (Korhonen and Martikainen, 1991), inactivation rates k_1 in northern surface waters ranged from 0.1 to 0.2 d^{-1}; temperature had little effect on the inactivation rates (Fig. 5). In warmer climates, values of k_1 are generally higher and the temperature effect is more pronounced (e.g., data of Flint, 1987 for *E. coli*, Mezrioui *et al.*, 1995, for *Salmonella* and *E. coli*, and Wright, 1989, for *Salmonella* and *E. coli* in Fig. 5). The lowest *E. coli* inactivation rate of 0.03 d^{-1} at the first inactivation phase has been reported for groundwater at temperatures from 4 to 6 °C (Cook and Bolster, 2007).

It has been suggested that the temperature dependence of the k_1 rate can be simulated with the Arrhenius equation, transformed to the form

$$k_1 = k_{1,20}\theta^{t-20} \tag{1}$$

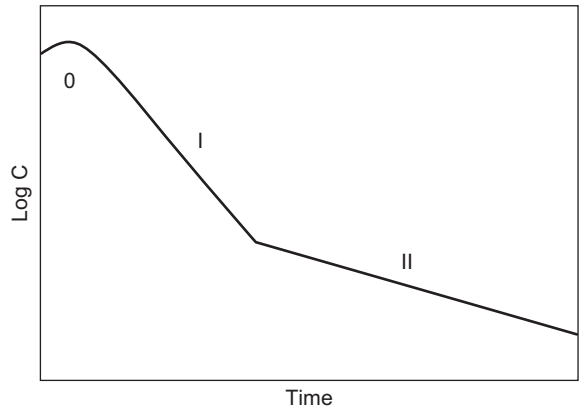

Figure 4 Three characteristic stages of the biphasic microorganism inactivation. Log C = Natural log of concentration.

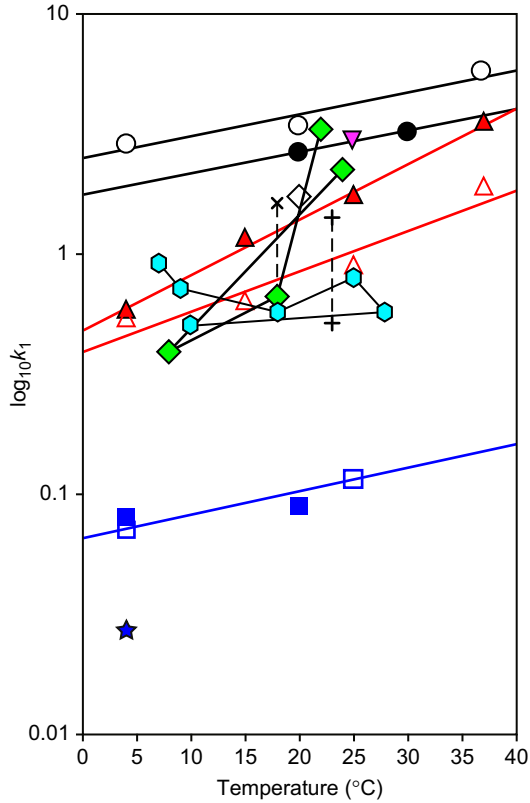

Figure 5 Examples of microbial inactivation rates at the first phase of inactivation and their dependencies on temperature; ○ Bogosian *et al.* (1996), single strain *E. coli* in non-sterile river water, Missouri; ● Chandran and Hatha (2005), *E. coli* in Cochin estuary water, India; ▽ Wright (1989), *E. coli*, tropical stream, Sierra Leone; ◇ Hellweger *et al.* (2009), single strain *E. coli*, sterile phosphate buffer solution; ▲ and △ Flint (1987), below and above the sewage outfall, respectively, single strain *E. coli*, river Sowe, England; – –×– – Dutka and Kwan (1980), range of k_1 values observed at four stations at the Lake Michigan; — + — Easton *et al.* (2005), k_1 values for *E. coli* O157:H7 (top symbol) and generic *E. coli* (bottom symbol); —◇— Mezrioui *et al.* (1995), *E. coli*, summer 85, autumn 85, winter 86, summer 86, *E.coli* single strain, stabilization pond system, Mediterranean; —⬡— Rhodes and Kator (1988), *E. coli*, Fox Mill Run stream, Chesapeake Bay, □ Korhonen and Martikainen (1991), single strain *E. coli*, a lake in Finland, ■ Budzińska *et al.* (2009), *Salmonella* Seftenberg, water from the Borowno Lake, Poland, ★ Cook and Bolster (2007), *E. coli* O157:H7, groundwater, south central Kentucky.

where t is time, $k_{1,20}$ is the rate of the exponential inactivation at 20 °C and θ is the temperature sensitivity parameter (Mancini, 1978). This equation can be transformed to

$$\log k_1 = \log k_{1,20} + (t - 20)\log\theta \quad (2)$$

that is, log k_1 has to be linearly related to the temperature if model (1) is applicable. Data in Fig. 5 illustrate the applicability of this model. The model (1) works reasonably well where all inactivation experiments are conducted in parallel using the water collected from the same site (e.g., data of Bogosian *et al.*, 1996, or Flint, 1987, in Fig. 5). However, model (1) does not work well when inactivation experiments are conducted with water from the same site but in different seasons (see data of Mezrioui *et al.* (1995) and Rhodes and Kator (1988); in Fig. 5, both datasets are shown with symbols connected with lines). Values of k_1 also varied significantly when measurements were made at the same time but at different locations (see data of Dutka and Kwan, 1980, in Fig. 5). Therefore, model (1) should be corrected for composition of water in different seasons. The proper correction factor is currently unknown.

The value of θ in Eq. (1) and (2) reflects the sensitivity of inactivation rates to temperature. It varies between 1.02 and 1.05 for data in Fig. 5. These numbers are substantially smaller than the value of 1.07 obtained by Mancini (1978) and used in watershed microbial modeling, for example, as the default value of the temperature adjustment factor (Parajuli *et al.*, 2009).

Nutrients are a substantial factor in determining bacteria survival in water. For example, differences in nutrient contents can explain variation in *E. coli* inactivation rates in river water above and below sewage outfall (data of Flint, 1987). McFeters and Stuart (1972) observed that *E. coli* could grow in autoclaved water taken below a sewage outfall but not above the sewage outfall, probably because of the differences in the nutrient content. Hutchison *et al.* (2005) reported an increase in the survival of *E. coli* O157: H7 when manure was added to water.

In some circumstances, the value of pH can be a substantial factor in *E. coli* survival. Whereas pH in the range from 5.5 to 8 did not affect *E. coli* survival, very low and very high pH substantially decreased survival (McFeters and Stuart, 1972). Sjogren and Gibson (1981) demonstrated that a bacterium such as *E. coli* exhibits an increased potential for survival in aqueous environments by utilizing a proton gradient generated by a lowering of the pH.

Sunlight is the most important inactivating factor in determining survival of fecal indicator bacteria, *E. coli* and pathogens such as *Salmonella* typhimurium in surface waters. Populations of *E. coli* and *S.* typhimurium decreased by 3.5 logs and 4.5 logs, respectively, under direct sunlight in waters of the Cochin estuary (Chandran and Hatha, 2005). The inactivation rate of *E. coli* under direct sunlight was about an order of magnitude larger than in darkness in an irrigation pond (Maïga *et al.*, 2009); the value of the k_1 rate constant was about 19 d^{-1}, which is much higher than anything shown in Fig. 5, which represents only experiments without direct sunlight. Inactivation of *E. coli* and enterococci declines with depth (Barcina *et al.*, 1990; Maïga *et al.*, 2009;

Murphy *et al.*, 2010). The majority of published data is on the survival of enteric bacteria in saline as opposed to fresh waters. Since the light absorbance of fresh waters is substantially higher than that of seawater, caution should be exercised when using survival data from elevated salinity environments.

An understanding of the mechanism(s) responsible for biphasic inactivation is currently lacking. The second phase (phase II in Fig. 4) can exhibit several behaviors relative to the first: faster decrease (e.g., Cook and Bolster, 2007), substantially slower decrease (Easton *et al.*, 2005), essentially stationary (e.g., Dutka and Kwan, 1980), or even an increase indicative of growth (Hellweger *et al.*, 2009). Some hypotheses have been put forward (Hellweger *et al.*, 2009), but have never been tested. Phenotypic variants could explain biphasic inactivation, with sensitive cells killed and more resistant cells selected and eventually growing slowly.

Indigenous biota of the water source can have multiple effects on microorganism survival. Both amoeba and algae have been shown to enhance the survival of *Campylobacter*, a water-borne pathogen that otherwise has a very fast die off (Axelsson-Olsson *et al.*, 2010). Algae have been shown to harbor substantial populations of *Salmonella* (Byappanahalli *et al.*, 2009) and *Salmonella enterica* and *E. coli* O157 have been reported to survive in protozoa, and be released from protozoal vacuoles as viable cells (Brandl *et al.*, 2005; King *et al.*, 1988). The water source ecosystem controls the level of nutrients and the degree of water transparency, and can prevent sunlight that causes inactivation (Dewedar and Bahgat, 1995). However, little is known about the water ecosystem as the environmental factor of indicator and/or pathogen microorganism survival.

Different pathogens and/or indicator organisms can have substantially different inactivation patterns in surface waters. For example, Easton *et al.* (2005) reported that *E. coli* O157:H7 was inactivated about two times faster than generic *E. coli*. *E. coli* have been reported to survive longer than either *Campylobacter jejuni* (Cook and Bolster, 2007; Korhonen and Martikainen, 1991) or *Streptococcus faecalis* (Dutka and Kwan, 1980), while *Y. enterocolitica* typically survived much longer than *E. coli* (Boyle *et al.*, 2008; Lund, 1996). In contrast, McFeters and Stuart (1972) reported that *Salmonella* survival was somewhat similar to that of *E. coli* in many natural settings. *Shigella spp.* have been found to survive longer than the usual indicators (McFeters *et al.*, 1974). In untreated river water, the die-off of *E. coli* and enterococci was approximately 10 times faster than die-off of oocysts, but die-off rates of *Clostridium perfringens* were lower than those of oocysts (Medema *et al.*, 1998). These results are probably just a snapshot of the capabilities that exist among different strains of each organism and dependent upon the source associated with the strains.

Responses of different microorganisms to environmental factors may also differ. For example *Salmonella* spp. populations exhibited significantly less die-off and stress than did *E. coli* at water temperatures of <10 °C

(Rhodes and Kator, 1988). In brackish water the survival rate of *S.* Typhimurium has been reported to be both higher (Mezrioui *et al.*, 1995) and lower (Chandran and Hatha, 2005) than that of *E. coli*. Die-off rates specific for a pathogen in question have to be used for environmental estimates and modeling. Corrections for the stress factors have to be introduced.

Overall, temperature, pH, availability of nutrients, and radiation are traditionally viewed as the leading factors of survival for both indicator and pathogenic microorganisms in natural waters and other environments. For a specific site, multiple regression equations can be developed to reflect the effect of these factors on survival (e.g., Fallowfield *et al.*, 1999). This indicates an opportunity for the eventual development of a process model of indicator and pathogen survival in irrigation waters, which currently does not exist. However, there are probably additional, but less obvious, factors important in selecting specific pathogen variants (cells) based on fitness in unknown environmental niches.

5.2. Importance of environmental microbial reservoirs for irrigation water quality

Irrigation systems are complex, and some of their compartments can serve as reservoirs where indicator and pathogen microorganisms can survive and multiply. The knowledge about these reservoirs is uneven. Whereas bottom sediments and bank soils have received substantial attention (Pachepsky and Shelton, 2011), aquatic biota as a pathogen reservoir in irrigation systems has only recently become a topic of research, and little if anything is known about biofilms in irrigation systems and drainage networks as pathogen reservoirs. Again, these are some additional factors that may select for different fitness characteristics among pathogens and relevant to water-related pathogen contamination of produce.

5.2.1. Bottom sediments

It has been known for some time that substantial populations of pathogenic and indicator microorganisms are harbored in freshwater bottom sediments. Testing sediments to evaluate bacterial pollution was first proposed more than 100 years ago (Savage, 1905). Geldreich (1970) characterized bottom sediments as a reservoir for fecal pollution 'fallout' from overlying water. However, the relative importance of sediments as bacterial habitats and as a source of water-borne fecal coliforms and *E. coli* was not recognized until recent publications describing that resuspension of sediments, rather than runoff from surrounding lands, can create elevated *E. coli* concentrations in water (Pachepsky and Shelton, 2011). Various pathogenic microorganisms have been detected in freshwater sediments, including bacteria such as *Mycobacterium avium* (Whittington *et al.*, 2005), *Salmonella* (van Donsel and Geldreich, 1971), *Clostridium botulinum* type E (Perez-Fuentetaja *et al.*,

2006), *Aeromonas hydrophila* and *Shigella* spp. (Obasohan *et al.*, 2010), and *Campylobacter* (Abulreesh *et al.*, 2006); protozoa such as *Cryptosporidium* (Searcy *et al.*, 2006a) and *Giardia* (Crokett, 2004); and various viruses (Miura *et al.*, 2009). Nevertheless, much less is known about pathogens in sediments as compared with indicator organisms in sediments (Cahoon and Song, 2009).

Extremely large variations have been recorded in microorganism concentrations in sediments from different sources as well as within a single stream or water body. Literature reports for values of *E. coli* concentrations in sediments vary from 1 to 500,000 MPN or CFU per g dry weight (Pachepsky and Shelton, 2011). Strongly asymmetric distribution functions have been found where replicate samples have been taken (Berry *et al.*, 2007). It is not uncommon to find two to five orders of magnitude differences between maximum and minimum concentrations observed at the same site or in the same watershed. In addition, bacterial concentrations in sediments may quickly decrease up to half order of magnitude per cm (Garzio-Hadzick *et al.*, 2010), with depth. Comparisons of microorganism contents in sediment and in the water column above it have inevitably led to the conclusion that sediments are the dominant reservoir for water-borne microorganisms. Numerous authors have observed that concentrations of fecal coliforms (FC) in sediment are multiple-fold higher than in the water column. Davies-Colley *et al.* (2004) analyzed data from agricultural streams in New Zealand and concluded that most of the time the water in the agricultural streams contained only a tiny fraction (about 1/1000) of the total FC contamination in the stream; the rest resided in the streambed from where it could be released by floods. Brandl (2006) noted that *E. coli* and *Salmonella enterica* survive well in water sediments, and seasonal flooding of fields with overflowing stream water has been suspected for *E. coli* O157:H7 outbreaks associated with leafy greens (Cooley *et al.*, 2007). Sediments and flooding are risk factors of potential crop contamination.

Correlations between *E. coli* and FC concentrations in sediment and in the water column are usually not significant. At any given sampling site, the measured bacterial concentrations are derived from multiple locations upstream of the sampling site. An additional factor is stream depth versus sampling depth. Even during periods of sediment re-suspension, if sediment-borne organisms are not distributed throughout the entire water column, water samples taken near the surface may not be representative of the total load. Finally, the diurnal oscillations in *E. coli* concentrations in stream water may be up to one and a half orders of magnitude (see Section 1). This difference may compromise correlations with *E. coli* concentrations in sediment, although nothing is known about the diurnal oscillations of *E. coli* concentrations in sediments.

High variability of concentrations in time is characteristic for sediment-borne bacteria. In fact, this variability in conjunction with resuspension is a

likely explanation for the erratic variations in indicator organism concentrations frequently reported in water quality monitoring (Chawla *et al.*, 2003; Giddings and Oblinger, 2004). Temporal variability is due to the relative rates of bacterial growth/die-off and to episodic re-suspension and re-distribution of sediments due to rainfall events, while spatial variability is due to stream bed heterogeneity. Seasonal dynamics in *E. coli* concentrations in sediments have been documented by several authors. Goyal *et al.* (1977) observed that the numbers in canal sediments were higher in winter than in summer, and attributed these differences to lower die-off rates in winter months. On the other hand, Crabill *et al.* (1999) encountered three orders of magnitude higher sediment FC concentrations in the summer versus the winter. The authors suggested the frequent flushing of sediments during the winter melt as a possible cause of the decrease of the sediment FC population in winter. Based on a large scale survey of the Rio Grande basin, Hartke *et al.* (2005) found no *E. coli* O157 in sediments during the summer; however, its prevalence varied from 0% to 80% in fall, winter and spring samples. The year-to-year variations in *E. coli* concentrations in sediment have been attributed to climatic conditions (Cinotto, 2005).

The spatial variability of *E. coli* and FC concentrations has often been attributed to the differences in sediment particle size distributions. Regression analysis has confirmed a significant direct relationship between the percentage of clay and silt particles and FC and *E. coli* concentrations in estuarine and riverine sediment samples in Northern California (Atwill *et al.*, 2007). Garzio (2009) observed an increase in sediment *E. coli* concentrations with increasing silt content in the sediment of a Maryland creek. The same trend was observed by Niewolak (1989) across 10 observation sites on a river in Poland. On the other hand, Cinotto (2005) reported the highest median concentration of *E. coli* in the 0.125- to 0.5-mm size range of natural sediments. The contradictory reports on the effect of sediment texture on the size of *E. coli* and FC populations are probably related to the multiplicity of ways in which the particle size distribution can affect the persistence of these organisms. Coarse sediments may not provide sufficient protection from the environment to allow the persistence of a substantial concentration of bacteria; for example, with too much exposure, bacteria may be subject to the effects of sunlight inactivation or protozoan grazing. On the other hand, availability of nutrients may be better in coarse sediments (Cinotto, 2005). Differences in sediment organic matter contents have been postulated as a possible explanation of the spatial variation in *E. coli* and FC populations, with mixed results. No correlation between organic matter content and total FC concentration was found in streambed sediments from a tropical rainforest (Buckley *et al.*, 1998). On the contrary, FC concentrations in the sediments were reported to be significantly higher in the presence of organic matter (Ferguson *et al.*, 1996).

Bacterial loads associated with human or animal presence and/or activities could, in some cases, be related to the elevated concentrations of *E. coli* in sediments. Crabill *et al.* (1999) observed that locations of creek recreational use (activity in water) coincided with increased concentrations of sediment-borne FC. This led the authors to the conclusion that recreational use served as the FC distribution system. Giddings and Oblinger (2004) suggested that the high *E. coli* densities at one of their sites was due to fecal contamination by domestic animal operations and home sites upstream, and the large area of sediment accumulation from upstream sources. The distance from the source of pollution affects concentrations of FC and *E. coli* in sediments when the source of fecal pollution is clearly defined. Goyal *et al.* (1977) observed an inverse relationship between FC in sediment and the distance from the sewage outfalls in the canals of the Texas West Coast. A similar strong dependence was documented by Haller *et al.* (2009) near the water treatment outlet at the Lake Geneva. Time spent by birds at the observation sites at the Chesapeake Bay strongly correlated ($r = 0.79$) with FC concentrations in sediments (Hussong *et al.*, 1979). FC numbers increased 100-fold in the sediments of water bodies following their colonization by water fowl in Poland (Niewolak, 1989).

A wide range of *E. coli* die-off rates in sediments have been reported in the literature. Interpretation of these data, however, is problematic, since, as previously discussed, die-off rates are highly dependent on the ability of introduced strains to adapt to and persist in specific sediment habitats. In an early study by van Donsel and Geldreich (1971), the authors reported a 90% die-off of both FC and *Salmonella spp.* in 7 days in various sediments. By comparison, in the study of Davies *et al.* (1995), 85 days was required to reach 90% FC die-off.

A common way to generalize data on FC and *E. coli* survival data in sediments is to use the exponential die-off model Eq. 1. The inactivation rates found in the literature vary significantly. The inactivation rates of *E. coli* in inoculated sediment from lakes of the Eastern United States were about 0.54 d^{-1}. Jamieson *et al.* (2004) reported a value of $k_1 = 0.15\ d^{-1}$ in sediments of Southern Ontario creeks. A FC inactivation rate constant of $0.07\ d^{-1}$ was published for sediment from Hillsborough River, FL (Anderson *et al.*, 2005). The biphasic die-off (Fig. 4) of *E. coli* in sediments was demonstrated by Garzio-Hadzick *et al.* (2010). Die-off rates strongly depended on temperature, particle size distribution, and organic matter content in sediment in this work.

E. coli survival has been observed to be longer in sediments than in the water column (e.g., Craig *et al.*, 2004). Inactivation rates in sediment can be an order of magnitude lower than those for the water column (Anderson *et al.*, 2005; Jamieson *et al.*, 2004). Decrease in temperature, increase in organic matter content, and increase in clay content, especially smectite clays, improve survival of bacteria in sediments. The effect that nutrients and pollutants in solution and sediment have on FC survival is uncertain.

Nutrients can both support growth of the bacterium in question (Jeng *et al.*, 2005) and cause an increase in competition and predation (Banning *et al.*, 2003). The presence of fine solid material devoid of biota may stimulate initial *E. coli* growth (Desmarais *et al.*, 2002; Laliberte and Grimes, 1982).

5.2.2. Resuspension and settling

Resuspension of sediments may account for much of the measured water-borne FC and *E. coli* during or shortly after rainfall events. The concentrations of *E. coli* and FC in streams during storm events are usually 2–3 orders of magnitude higher than in the baseflow conditions (e.g., Hunter *et al.*, 1992). At least three resuspension mechanisms can act during the high flow events (Wilkinson *et al.*, 2006). A steep-fronted wave, with wave height much greater than the preceding water depth, can effectively suck organisms from the bottom sediment and hold them in the turbulent wavefront. A less steep front or falling wave can lift organisms but not draw them in the wave overrun. Finally, the steady-flow stochastic erosion of bed and/or bank sources, resulting from high flow turbulence, can maintain FC concentrations elevated above those encountered at lower rates of flow. Davies-Colley *et al.* (2004) described storm chasing studies that showed that FC pollution in streams typically peaks well ahead of stream flow peaks. This timing reflects bacteria being resuspended from sediments by accelerating currents rather than subsequent wash-in.

The increase of *E. coli* concentrations in a water column has been noted due to sediment resuspension, which is unrelated to rainstorms. Phillip *et al.* (2009) recorded a fourfold increase in mean *E. coli* densities and a threefold increase in total suspended sediments in the water column due to the recreational wading in a small tropical stream. Recreational boating has been implicated in elevated *E. coli* concentrations in lake water (An *et al.*, 2002). Irrigation water intake can cause substantial resuspension of sediment (Paulos *et al.*, 2006) and become a possible reason for transport of microorganisms to fields.

Association of microorganisms with sediment particles in soils is often assumed to be a function of the clay content. One available empirical equation for *E. coli* is $K_d = 10^{-1.6 \pm 0.9}\text{CLAY}^{1.98 \pm 0.7}$ where K_d is the partition coefficient, that is, the ratio of the associated concentration in cell g^{-1} and concentration in water cell mL^{-1}, CLAY is the percentage of clay particles < 0.002 mm in soil, $2 < \text{CLAY} < 50$ (Pachepsky *et al.*, 2006).

The schematic subdivision of resuspended bacteria into free-floating versus attached to individual solid particles may be misleading. Bacteria frequently persist in the environment in heterogeneously distributed microcolonies or biofilms. Very little is known about the relative stability of sediment biofilms. Pettibone *et al.* (1996) observed that a few large flocculated particles accounted for most of the volume of resuspended sediment-borne FC in a study conducted with waters from the Buffalo River before and after ship passage. Resuspension of flocculated, bacteria-laden particles affects bacteria transport

and deposition as well as survival. Sediment flocs can be a dominant form of sediment transport in freshwater fluvial systems (Droppo and Ongley, 1994).

Since bacteria are small and light, their settling rates are extremely low (1.6 m d^{-1} as estimated by Cizek *et al.* (2008)). However, settling rates of sediment-associated bacteria are significantly higher due to the density of sediment particles (Gannon *et al.*, 1983). The straightforward representation of settling in streams based on Stokes law, that is, as the pure effect of the effects of viscosity and gravity, has been recently questioned. Cooley *et al.* (2007) indicated that the hyporrheic exchange, that is, exchange through the subsurface volume of sediment and porous space adjacent to a stream, can lead to high rates of suspended particle deposition in sediment beds, even when the suspended particles are very small and have no appreciable settling velocity. On the other hand, Jamieson *et al.* (2005) reported that the calibrated settling velocities observed in their study were two orders of magnitude lower than the corresponding Stokes fall velocities. They suggested that the high shear stresses occurring near the bed limited the number of particles that can actually bond to the bed without being re-entrained.

Recently, modeling of bacteria release from sediment has been included in pathogen fate and transport modeling in streams and lakes (Bai and Lung, 2005; Cho *et al.*, 2010; Jamieson *et al.*, 2005). Modeling showed that an unrealistic surface input of *E. coli* may be needed to explain the dynamics of *E. coli* concentrations if the sediment resuspension is ignored (Kim *et al.*, 2010). None of the existing models quantifies *E. coli* exchange between water and sediment due to sediment resuspension; the rate and impact of this process remain essentially unknown. Similarly, modeling of *E. coli* persistence in sediment has begun only recently (Rehmann and Soupir, 2009). The presence of bottom sediments containing large, unquantified reservoirs of fecal pollution indicator bacteria introduces substantial uncertainty in detection, monitoring, and control of microbiological water quality and stream impairment.

5.2.3. Bank soils

Bank soils present yet another reservoir of microorganisms that can affect microbial water quality. Autochthonous (indigenous) *E. coli* have been found in soils of various environments, first in tropical (Byappanahalli and Fujioka, 1998), then in subtropical (Desmarais *et al.*, 2002; Solo-Gabriele *et al.*, 2000) and finally in temperate regions (Byappanahalli *et al.*, 2006). In the latter work conducted with soils of several coastal Lake Superior watersheds, PCR-based DNA fingerprint analyses indicated that there was a 92% similarity/relatedness in *E. coli* genotypes that survived over winter in frozen soil. Growth at high temperatures (30–35 °C) and survival at medium temperatures (25 °C) were also observed in this work.

The distance from the water edge affects the density of *E. coli* populations in soils and beaches. Byappanahalli *et al.* (2003) noted that *E. coli* concentrations decreased rapidly with distance from the streambed.

A similar pattern was reported by Desmarais *et al.* (2002) who observed 1 log difference in *E. coli* concentrations in soil at a distance of 90 cm from the edge of the water.

E. coli appears to be able to utilize constituents of the soil organic matter for population support and growth (Tate, 1978). However, the presence of organic litter does not necessarily imply elevated populations of *E. coli*. Byappanahalli *et al.* (2003) surveyed banks of a stream in Michigan and found that *E. coli* was highest in relatively clean moist sands and much lower in litter-laden sandy soils.

Soil water content affects the survival and regrowth of *E. coli*. The greatest survival of coliforms was noted with anaerobically grown cells amended to flooded soil as compared to moist soil in the work of Tate (1978). However, temporary drying did not eliminate the *E. coli* population in soil taken at 50 cm distance from the edge of water (Desmarais *et al.*, 2002). Laboratory experiments of Solo-Gabriele *et al.* (2000) confirmed that, upon soil drying, *E. coli* is capable of multiplying by several orders of magnitude. The authors hypothesized that *E. coli* can survive at lower soil moisture than its predators, and therefore, upon soil drying, conditions are suitable for *E. coli* growth. Under this assumption, it is likely that the outer fringes of the channel banks, which experience the most extreme drying conditions, dominate the contribution of *E. coli* to the water column.

All surface sources of irrigation water include bottom sediments and bank soils as reservoirs of microorganisms. These reservoirs affect water transported in irrigation canals and ditches. Water from subsurface sources or wastewater treatment plants is often stored in presence of sediments before being dispensed to fields. Available data show that bottom sediments and bank soils may have substantial effect on microbiological quality of irrigation waters.

5.2.4. Aquatic biota

Aquatic ecosystems include a host of biota in addition to microorganisms. Some of these, in particular algae and amoebae, can affect growth of pathogenic bacteria. Numerous studies suggest that bacteria and algae coexist in an association that benefits both groups of organisms (e. g., Carr *et al.*, 2005). Cinotto (2005) studied an impoundment area in a Pennsylvania creek and observed that elevated aquatic growth resulted in sharp increases in *E. coli* concentrations from upstream to downstream throughout the impoundment area for 2 years in a row. In laboratory experiments by Ksoll *et al.* (2007), *E. coli* readily colonized periphyton (the mixture of algae, microbes, and detritus attached to surface) from the Lake Superior shoreline and persisted for several weeks; in addition, cells were released to the overlying water. Field data of these authors showed a significant linear relation between fecal coliform concentrations and periphyton ash-free dry weight. The bacterial pathogens *Shigella*, *Salmonella*, *Campylobacter*, and

shiga toxin-producing *E. coli* (STEC) were recently found to be associated with *Cladophora*—a green alga growing in southern Lake Michigan (Byappanahalli *et al.*, 2009). Aquatic flora in a sewage-impacted area of the southern Asian Gangetic riverine system exhibited significant high levels of enterotoxigenic *E. coli* (Singh *et al.*, 2010). Algae can enhance survival of microorganisms in surface and subsurface water sources in various ways, including the release of carbon substrates (Carr *et al.*, 2005), protection from direct and indirect sunlight (Dewedar and Bahgat, 1995), and attachment to plant surfaces (Karim *et al.*, 2004).

Amoebae have been shown to host a wide variety of bacterial species including many human pathogens such as *Vibrio cholerae*, *L. monocytogenes*, *Mycobacterium* spp. and *Helicobacter pylori* (Thomas *et al.*, 2009). In addition to warm blooded hosts, *Campylobacter* spp. might use water bound organisms as hosts for survival and potentially for replication in the environment (Axelsson-Olsson *et al.*, 2010). Pathogens have been transported by zooplankton (Grossart *et al.*, 2010), and by other aquatic organisms.

Although the ability of aquatic organisms to facilitate survival of pathogens and indicator organisms is proven, the relative importance of water biota as the reservoir of pathogen and indicator organisms in irrigation systems is currently unknown. Additional studies are needed to (i) document the occurrence of invertebrate-associated pathogens in relevant field conditions, such as distribution systems; (ii) assess the fate of microorganisms ingested by higher organisms in terms of viability and (or) infectivity; and (iii) study the impact of internalization by zooplankton on pathogen resistance to water disinfection processes, including advanced treatments such as UV disinfection (Bichai *et al.*, 2008).

5.2.5. Biofilms in pipe-based irrigation water delivery systems

There is currently not a universally recognized definition for biofilms. However, it is commonly agreed that a water distribution system biofilm consists of a complex mixture of microbes, organic, and inorganic material accumulated amidst a microbially produced organic polymer matrix attached to the inner surface of the distribution system (U. S. EPA, 2002).

Biofilm formation in irrigation systems is a well known phenomenon. In particular, there has been a lot of attention paid to biofouling and clogging of drip-irrigation emitters (Cararo *et al.*, 2006; Dehghanisanij *et al.*, 2004; Ravina *et al.*, 1992; Taylor *et al.*, 1995). Several recent studies have revealed the potential for biofilms within drinking water distribution systems to harbor pathogenic bacteria (e.g., September *et al.*, 2007) and viruses (e.g., Skraber *et al.*, 2005). However, no peer-reviewed research has been reported on biofilms as potential reservoirs of pathogenic microorganisms in irrigation distribution systems. Potential effects of biofilms on irrigation water quality can be inferred from the literature on pathogens in biofilms in drinking water distribution systems and from the literature on food safety. It has been

demonstrated for drinking water systems that practically any microbe (including some pathogens) present in water may attach, or become enmeshed, in the biofilm. Pathogens, which cause disease in healthy humans, may persist in the biofilm. However, the survival time for many pathogens in biofilms is uncertain and likely varies depending on the organism. Biofilms may extend the survival of human and zoonotic pathogens by protecting them from disinfectants. These pathogens may be sloughed from the biofilm into the water column due to changes in the flow rate (U. S. EPA, 2002).

Biofilms may present a reservoir for pathogenic protozoa in their nonreproductive protective stage. Searcy *et al.* (2006b) observed the capture and retention of *C. parvum* oocysts in *Pseudomonas aeruginosa* biofilms using laboratory flow cells. Given the ability of *Cryptosporidium* oocysts to adhere to fresh produce (Macarisin *et al.*, 2010) and the common presence of *C. parvum* oocysts in many sources of irrigation water (Thurston-Enriquez *et al.*, 2002), biofilms as reservoirs of *C. parvum* oocysts in irrigation systems should be examined.

In addition to the well established water- and food-borne pathogens, infections may also be caused by other common microbes, referred to as opportunistic pathogens. Opportunistic pathogens include *Pseudomonas aeruginosa*, *Legionella pneumophila*, and the *Mycobacterium avium* complex (MAC). These microorganisms have attracted attention with respect to biofilms in drinking water system, because they are well-adapted to the low nutrient level and cool water temperature of water distribution systems (Lau and Ashbolt, 2009). These organisms can also survive in produce (e.g., Morris and Monier, 2003), and therefore, the importance of these microbes populating biofilms in irrigation systems should also be evaluated.

One consequence of pathogen accumulation in biofilms in irrigation pipes is the loss of indicator organism utility. Irrigation pipe biofilms may compromise the effectiveness of microbial indicators of water quality in two major ways (Geldreich, 1996). Firstly, incorporation of indicator organisms into pipe biofilms will result in higher concentrations at intake versus the actual irrigation stream. Secondly, because coliforms can grow and detach into the flowing water, concentrations may increase versus the intake.

The following factors have been demonstrated to affect pathogen survival and growth in drinking water distribution systems: (a) environmental factors, (b) presence of nutrients, (c) microbial interactions, (d) pipe material, (e) system hydraulics, (d) disinfectant type and residuals, and (e) sediment accumulation (U. S. EPA, 2002). All these factors undoubtedly can affect presence of pathogenic microorganisms in biofilms developing in pipe-based irrigation water delivery and distribution systems. The effects of environmental factors such as temperature and pH are probably organism-specific, and may depend on whether organisms attach and actually form biofilms, or adhere to an existing biofilm. LeChevallier *et al.* (1987) found a positive correlation of heterotrophic bacteria counts with both

increased temperature and pH. Attachment of *Salmonella* enteritidis to stainless steel surfaces was larger at 20 °C compared with 5 and 37 °C (Giaouris *et al.*, 2005). Patel *et al.* (2011a) observed significantly higher attachment of *E. coli* O157:H7 on a spinach harvester blade when incubated at simulated temperature of California (30 °C-day, 20 °C-night) than at 22 °C. On the other hand, a temperature increase in the range from 13 to 42 °C led to a decrease in the attachment of *Campylobacter jejuni* to stainless surfaces (Sanders *et al.*, 2008). Temperature affected not only attachment but also the detachment of this bacterium to biofilms (Nguyen *et al.*, 2010). The probability of detachment was significantly higher at 4 °C than at 25 °C for five of the six strains tested.

The presence of nutrients enhances biofilm growth with carbon usually being the limiting factor (Mains, 2009). An influx of nutrients may not be beneficial for survival of a specific organism in a biofilm. Banning *et al.* (2003) observed reduction in *E. coli* survival with the increase in available nutrients and attributed that to either enhanced competition for nutrients or enhanced antagonism by the indigenous microbial population.

The ability of pathogens to form biofilms may depend on the composition of the microbial population. Klayman *et al.* (2009) reported that *E. coli* O157:H7 required *Pseudomonas aeruginosa* as a colonizing partner to adhere and persist in a capillary flow cell. Habimana *et al.* (2009) observed that the initial attachment of *L. monocytogenes* to biofilms was dependant on the genetic background of bacteria that formed the biofilm; some biofilms reduced the ability of *L. monocytogenes* to attach.

Pipe material can be a strong factor controlling biofilm formation. Metal pipes present better substrates compared with PVC pipes (e.g., Silhan *et al.*, 2006), although the release of organic components from PVC pipes may contribute to biofilm persistence at later stages. Sediments, or loose deposits, have been claimed to harbor pathogens in drinking water systems (Rubulis *et al.*, 2008). Other components can also support biofilm growth including materials used in valves, gaskets, washers, pump lubricants, and pipe coatings (Mains, 2009).

The effects of hydraulic regime on biofilm development and pathogen survival in water distribution systems varies widely. Extreme flow regimes appear to cause substantial changes, for example, low velocities may result in stagnant water, which facilitates microbial growth (Reynolds *et al.*, 2008; Völker *et al.*, 2010). High water velocities may increase the level of nutrients in contact with the biofilm, on one hand, and also cause greater shearing of biofilms that may contain pathogens from the pipe surface, on the other hand (O'Toole *et al.*, 2000). The complexity of drinking water delivery systems causes differences in opportunities for biofilm formation in different parts of the pipe network. This also may be true for irrigation water delivery systems, although they presumably have simpler configurations than drinking water systems. Although biofilm growth and shear removal has been

simulated in simple pipe rigs (Eisnor and Gagnon, 2003; Maier, 1999), a simple relationship between the hydraulic effects and microbial growth in biofilms has not been reported or does not exist (U. S. EPA, 2002).

All factors of biofilm formation and microorganism survival in biofilms found in drinking water distribution systems are represented in irrigation water delivery systems. However, these two types of pipe-based water delivery systems have quite different parameters, including temperature and pH, nutrient contents and proportions, microbial communities, pipe and sediment material, hydraulic regimes, and disinfection techniques. The biofilm formation from creek water has been shown to exhibit seasonality that could not be explained by any measured water quality parameters (Wolyniak *et al.*, 2010). Therefore, the knowledge base on biofilms accumulated in works for drinking systems conditions has to be evaluated with respect to its applicability for irrigation systems. Currently, irrigation systems lack any credible information on biofilm formation and pathogen survival and release in such biofilms.

6. Management and Control of Produce Contamination with Pathogens from Irrigation Waters

Several strategies have been proposed to reduce the risk of produce contamination with pathogens during irrigation. Figure 3 provides an overview of possible directions for irrigation water quality control. A decrease of pathogen inflow from direct input sources (runoff, direct deposition, infiltration and lateral flow in shallow soils, sewage discharge) and/or from pathogen reservoirs (bottom sediment, bank soils, algae, and periphyton) presents a possible strategy for microbiological water quality control. Treating water during storage, between storage and delivery systems and water while in the delivery systems represents another class of strategies. Changing the irrigation method may affect the pathogen availability to plants. Finally, manipulating irrigation schedules and concurrent use of irrigation waters of different quality can help to reduce the risk of produce contamination with pathogens.

Decreasing direct inputs of pathogens to irrigation water sources can present practical ways to improve the irrigation water quality. Estimates show that, at least in some cases, the pathogen input from wildlife can be much smaller than that from cattle and other domestic animals (Kim *et al.*, 2010). In the latter case, improvement of microbiological water quality may occur due to preventing animals from entering streams by fencing (Miller *et al.*, 2010) or using off-stream water sources - setting water troughs in riparian areas (Clawson, 1993), being aware of upstream use and sources of

water that are planned to be used in irrigation (Cooley *et al.*, 2007), and preventing manure runoff from fields, pastures, and feedlots to irrigation water sources as a part of good agricultural practices. Constricted wetlands have been shown to be efficient in decreasing fecal indicator concentrations in agricultural return flows that exceeded Californian standards for water discharge into state waterways (Diaz *et al.*, 2010). It has been noted that contradictions may arise between goals of keeping pathogens away from irrigation water by removing buffer strips that harbor wildlife populations and preserving water resources from impairment due to runoff and erosion (Crohn and Bianchi, 2008), and those may need to be reconciled.

Animal husbandry practices can affect the amount of pathogens in manure and possibly mitigate the microbial pollution of irrigation waters from runoff. For example, management practices have been recommended that limit the probability that feedlot cattle shed *G. duodenalis* in their feces (Hoar *et al.*, 2009), the duration of *Salmonella* shedding and dissemination by the manure removal methods (Kabagambe *et al.*, 2000), dietary interventions affecting *E. coli* O157 shedding in cattle (Jacob *et al.*, 2009), etc. Unless runoff enters environmental reservoirs suitable for pathogen growth, the decrease in their concentrations in runoff will decrease the risks of produce contamination.

Treating water during storage employs inexpensive methods. In warmer regions, waste stabilization ponds, waste storage and treatment reservoirs, on-farm sedimentation ponds, and filtration through sands and soils are widely used (Keraita *et al.*, 2010) and some of them have been proven to decrease the populations of pathogens (Maïga *et al.*, 2009 Mara and Silva, 1986;). In temperate regions, a similar technology combined with mixing was shown to be promising - if a shallow reservoir was well mixed to prevent gradients in temperature and dissolved oxygen, storage of irrigation water for a period of at least 2 weeks would reduce bacterial numbers by at least 3 logs during the growing season in Nova Scotia (Murphy *et al.*, 2010).

Eliminating or avoiding environmental reservoirs of pathogens at the irrigation water intake can be very efficient. Since sediments are substantial microbial reservoirs (see Section 5.2.1), water intake without disturbing sediments has the potential to improve the microbiological quality of irrigation waters (Ensink *et al.*, 2006; Keraita *et al.*, 2010). Algae and periphyton can serve as microbial reservoirs (Section 5.2.3); as a result, some GAPs (Good Agricultural Practices) include elimination of litter and algae in ponds used as an irrigation water source (Anonymous, 2010a).

Several technologies have been recommended for disinfecting water after it is taken from storage and before it is sent through the water delivery system to plants. For example, filtration, oxidation reduction, chlorination, ozonation, exposure to ultraviolet light, electronic beam processing, and heat treatment, or "heat pasteurization" all can potentially reduce the levels of microorganisms in irrigation water (Newman, 2004). The costs of the

treatments may vary (U. S. EPA, 2010) and can be prohibitive. Greenhouses and nurseries are primary adopters of those technologies. The evaluation of the site-specific applicability of any of those methods should be concerned about maintenance costs, safety, and biological effects on crops and humans, among other things. For example, chlorine is the disinfecting agent most commonly used for combined sewage overflows and it decreases turbidities to a level similar to that of irrigation water. However, the effectiveness of chlorine is reduced in water with high levels of organic matter; chlorine can react with organic matter to yield potential carcinogens, and the long-term effects of chlorine and other disinfectants on crops and soil are unknown (Steele and Odumeru, 2004).

Biofilm control in irrigation water delivery systems is currently an uncharted territory. Although little doubt exists about pathogen- and indicator-populated biofilm developments in pipes and other parts of irrigation systems, the efficiency of measures to control biofilms in these systems is unknown. Successful biofilm control programs incorporate multiple approaches such as system flushing, line replacement, nutrient level reduction, possible combinations of several disinfection methods, corrosion control, and filtration (Mains, 2008). It remains to be seen how these components can be selected and arranged in an efficient program to prevent the persistence of biofilms as pathogen reservoirs in irrigation systems.

Concurrent use of irrigation waters of different quality appears to be efficient when the availability of good-quality irrigation water is limited. The high quality water is directed to irrigate produce whereas the low quality water is used for forage crops. This approach, sometimes called crop restriction, has been recommended and successfully tried in Canada (Anonymous, 1988), USA (Anonymous, 1992), Mexico (Cifuentes *et al.*, 2000), and Chile (Westcot, 1997). Certification programs have been suggested to provide produce labels indicating that it was produced under safe conditions as a means of avoiding low compliance rates in crop restriction programs (Anonymous, 2002). Steele and Odumeru (2004) suggested that an alternative to crop restriction can be using water of low and high quality in the beginning and at the end of the growing season, respectively. They indicated that caution should be exercised when using this approach, because data regarding survival patterns of pathogens in soils and plants are not definitive.

The interval between final irrigation and harvest influences the extent of contamination, as pathogens have been shown to decline with time following cessation of irrigation before harvest. Proposed first for developing countries (Shuval *et al.*, 1986), the practice of irrigation cessation prior to harvest has become a popular recommendation in developed countries. A report to the UK Food Safety Agency indicated that "in the context of this review of food safety issues, the most important factors relating to irrigation scheduling are the amount of water which may be applied to a particular

crop, and the interval between the final application and harvest." The amount of irrigation will influence the potential loading of pathogens on the crop in the event that pathogens are present in the water. The 'harvest interval' indicates the time available for natural die-off of pathogens to occur (Groves *et al.*, 2002). The Quantitative Microbial Risk Assessment for irrigation waters (Section 4.3) uses the harvest interval as the essential input variable. However, knowledge about the effect of harvest interval on the produce microbial contamination is far from sufficient and should include several site-specific variables. Keraita *et al.* (2007) concluded that the measure can significantly reduce fecal contamination of lettuce during the dry season, but it is not suitable for the wet season due to favorable conditions for pathogen survival and re-contamination by splashes from contaminated soils.

The irrigation method may affect the pathogen availability to plants (Fonseca *et al.*, 2011). In particular, subsurface drip irrigation was shown to have potential in decreasing health risks when microbial-contaminated water was used for irrigation (Enriquez *et al.*, 2003; Song *et al.*, 2006) although it was not always the case (Moyne *et al.*, 2011). These reports relate to studies done in the arid zone, and a comparison of transmission of pathogens from irrigation water to vegetables with different types of irrigation is needed. The use of drip irrigation is limited with irrigation water with high turbidity, and other water delivery methods, for example, surface irrigation, may be of more benefit (Solomon *et al.*, 2002a).

Overall, a number of strategies have been proposed and tested to control the microbiological quality of irrigation waters. Monitoring of microbiological water quality is the essential part of application of any strategy or combination of strategies for minimizing produce contamination. Definitive site-specific data showing the effectiveness of interventions combined with incentives for their adoption will improve the microbiological quality of produce irrigation.

7. Research and Development Needs

Previous chapters demonstrate that, given the current level of the public awareness about the risks of microbiological produce contamination by irrigation water, the state of knowledge and regulations in this field are far from satisfactory. Producers, regulators, and consumers simply do not have the information to make informed decisions about site-specific risks of microbiological contamination of produce.

The sanitary quality of many irrigation water sources is unknown currently (Suslow, 2010). Moreover, how to determine and characterize the microbiological quality of irrigation water sources are subjects of recurrent debate. A monitoring protocol is needed that is based on the actual spatial

and temporal variability of pathogen and indicator concentrations in irrigation water sources and in environmental reservoirs. Some research supports monitoring pathogen and indicator organism concentrations in watershed sediments, periphyton, and algae based on the adherence of bacteria to flocs that provide nutrients and protection in aquatic systems and migration to the bottom of water columns (Droppo *et al.*, 2009). Irrigation system-wide monitoring has to be designed based on the fact that even water that is clean at the source can become microbiologically polluted as it is stored and distributed. The site-specific value of monitoring indicator organisms accepted currently for assessing risk of encountering key foodborne pathogens (such as *E. coli* O157:H7 and *Salmonella*) needs to be re-examined (Suslow, 2010). Suitable indicator organism(s) that relate to presence of pathogens in irrigation water need to be investigated. Where irrigation of leafy greens occur with well water, for example, in Salinas Valley, California, a monitoring study of suspect wells is needed with large volumes of water tested to identify whether there are dynamic pulses of bacteria, possibly fecal indicators, that reflect recharging of the aquifer with inferior water or some other factor. If pulses occur infrequently, they will be missed when a low volume of water is tested.

The current focus on zoonotic pathogens and indicators of their presence appears to be insufficient and may be misguided in some cases. In particular, presence of other microorganisms such as opportunistic pathogens such as *Legionella*, *and Mycobacterium*, in irrigation water sources and irrigation systems may present substantial health risks. Currently, little is known about the occurrence of those pathogens in irrigation water and their epidemiology as related to consumption of irrigated produce. Amoebas are another class of organisms that have recently been proven to be important for microbial water quality (Bukhari *et al.*, 2010) and are expected to be present in surface water used for irrigation. No methods to measure populations of those organisms have been validated for irrigation water sources.

Nothing is published about biofilms being pathogen and indicator microorganism reservoirs in irrigation water delivery systems. This is a critical issue given the role biofilms have been found to play in microbiological contamination of drinking water distributing systems. Biofilms protect embedded microbes against disinfection and most bacteria in water systems are attached to piping and other surfaces in the form of biofilms (Lazarova and Manem, 1995). Methods of biofilm detection in irrigation water delivery systems, practices to minimize their formation, and evaluation of the comparative efficiency of different disinfection technologies with regard to biofilms in irrigation equipment should be developed or adapted from other water system fields and validated.

Disinfection methods for irrigation systems have not been rigorously evaluated, and the remediation costs for contaminated water prior to

irrigation are listed as a knowledge gap (Suslow, 2010). Simple and inexpensive methods for improving the microbial quality of marginal irrigation water at the farm level need to be developed, tested, and demonstrated.

The risk assessment methodology for produce irrigation from sources other than wastewater has not been developed. Whereas Quantitative Microbial Risk Assessment is the accepted technology to evaluate risks of the disease transmission via irrigation waste waters and produce (Mara, 2010), irrigation from surface water sources relies mostly on indicator-based standards that have hardly any factual support and are not site-specific. Application of quantitative microbial risk assessment is needed to better understand the impact of low level pathogen transmission (Gerba, 2009).

Current assessments of microbiological quality of irrigation waters do not use any microorganism fate and transport modeling. Modeling can provide valuable insights given the current state of knowledge about irrigation water quality. Process-based models can provide reasonable estimates in absence of site-specific data, screen, and evaluate various management practices with respect to their relative efficiency, and estimate the uncertainty in microbial concentrations of source waters that can be used in quantitative microbial risk assessment. Empirical models of regional and local relationships between weather patterns, pathogen concentrations in waters and disease rates (e.g., Haley *et al.*, 2009) can be useful for predictive purposes as it is currently done for climate-sensitive diseases (Rogers *et al.*, 2002).

Some emerging issues in microbial quality of irrigation water may change the paradigm of preharvest produce safety and require increased attention. The ecology of microorganisms in irrigation water sources and in colonized plants may be relevant to understanding microbial contamination by irrigation of produce (Critzer and Doyle, 2010). Microbial community composition may be a better indicator of pathogen presence and survival in irrigation waters and in environmental reservoirs, as it has been in drinking water (Berry *et al.*, 2006; Bichai *et al.*, 2008). Climate change will affect the water resources availability and structure, and therefore will influence the microbiological safety of produce. A basic understanding of these factors currently is lacking.

Overall, there is a recognized need to establish HACCP-based produce safety standard protocols for irrigation of fresh produce. Standards need to be developed for irrigation waters that are meaningful and reduce the risk of produce contamination taking into consideration the means of irrigation and the type of produce (Gerba, 2009). Substantial knowledge gaps exist, and a concerted effort by researchers and practitioners is needed to maintain food safety of fresh produce in an increasingly intensive food production system and limited and declining irrigation water resources.

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