Fecal Contamination in the Wastewater Irrigation System and its Health Threat to Wastewater-Based Farming Households in Addis Ababa, Ethiopia

Environmental Health Insights
Volume 17: 1–12
© The Author(s) 2023
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/11786302231181307

Adane Sirage Ali[1,2], Sirak Robele Gari[1], Michaela L Goodson[3], Claire L Walsh[4], Bitew K Dessie[5] and Argaw Ambelu[1]

[1]Division of Water and Health, Ethiopian Institute of Water Resources, Addis Ababa University, Addis Ababa, Ethiopia. [2]Department of Environmental Management, Kotebe University of Education, Addis Ababa, Ethiopia. [3]Newcastle University Medicine Malaysia, Iskandar Puteri, Johor, Malaysia. [4]School of Engineering, Newcastle University, Newcastle upon Tyne, Tyne and Wear, UK. [5]Water and Land Resource Centre, Addis Ababa University, Addis Ababa, Ethiopia.

ABSTRACT: Due to rapidly growing demand, the production of vegetables is increasing along the Akaki Rivers. The objective of this study was to examine the degree of fecal contamination and levels of fecal contamination and dissemination throughout the wastewater irrigation system. Irrigation water, irrigated soil, and leafy vegetables were collected twice during 2 vegetable growing seasons, at the maturity period of the growing season, from 19 sampling points along the 2 Akaki Rivers. Composite samples were taken from all sampling points and *E.coli* was enumerated. The mean *E.coli* load in wastewater and non-wastewater sources were $1.1^{6}\pm5.5^{3}$ CFU/100 ml and $2.23^{2}\pm1.29^{2}$ CFU/100 ml respectively. All counts of *E. coli* in the wastewater exceeded the WHO's standards indicating that the irrigation water quality was unacceptable. In the wastewater-irrigated and non-wastewater-irrigated soil, the mean *E.coli* were $3.6^{2}\pm1.58^{2}$ CFU/g and $1.32^{2}\pm87.1$ CFU/g respectively. Meanwhile, the mean *E.coli* counts on the lettuce and Swiss chard were 78 ± 2 CFU/g and 44 ± 3 CFU/g respectively. The *E.coli* count on the leafy vegetables was found to be associated with the *E.coli* in the wastewater and soil. The production of leafy vegetables using wastewater with unacceptably high levels of *E.coli* and high occupational exposure introduces high levels of risk to the farming communities and to the consumers. Leafy, low-growing raw edible vegetables need careful treatment during food production and harvesting procedures or activities.

KEYWORDS: *E.coli*, wastewater irrigation, soil, vegetables, fecal contamination, lettuce

RECEIVED: February 25, 2023. **ACCEPTED:** May 24, 2023.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was partly supported by the Water Security and Sustainable Development Hub, which is funded by the UK Research and Innovation's Global Challenges Research Fund (GCRF), Grant no.: ES/S008179/1.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Adane Sirage Ali, Department of Environmental Management, Kotebe University of Education, Addis Ababa, 31248 Ethiopia. Email: utopia4eth@gmail.com

Introduction

The reuse of wastewater for agricultural irrigation is widely used around the world in treated or untreated forms.[1,2] Approximately 1.5%-6.6% of the global irrigated areas, which grow 10% of the world's crops, are irrigated by treated wastewater in developed nations.[2] Using untreated wastewater for agriculture is becoming popular in developing countries, particularly in regions where freshwater is scarce.[2] In Sub-Saharan Africa, about 10% of the population in cities is involved in the practice of wastewater irrigation; in West Africa, 50 to 90% of urban dwellers reported consuming vegetables irrigated with wastewater or polluted surface water.[3] The reuse of wastewater for agricultural irrigation is valuable for farmers because of its high nutrient content, reducing the cost of chemical fertilizers and increasing productivity.[4] In developing regions, besides being a source of water and nutrients, re-using wastewater helps to control pollution and tackle the challenge in food production.[4] Wastewater reuse may reduce the nutrient loads from wastewater discharges into different waterways, thereby reducing and preventing pollution.[5]

Nevertheless, the use of wastewater for crop production represents a potential public health hazards including severe health risks and contamination of drinking water sources, agricultural land, and crops with toxic metals, parasites, and microbial pathogens. Among the most important health problems, pathogenic microorganisms (viruses, protozoa, and bacteria) are the most pervasive, and are known to cause a wide range of diseases in a human beings.[6] Among these pathogens, foodborne bacteria capable of causing severe gastrointestinal diseases including *Salmonella* spp., *Klebsiella* spp., *Enterobacter* spp., *E. coli* O157:H7, and *Listeria monocytogenes* are found in irrigation water and agricultural soil.[7] Fecal pathogens can be transmitted via multiple routes to reach human beings and cause infections. Several outbreaks of human gastroenteritis associated with the consumption of raw vegetables have been reported in Canada, China, Nigeria and Ghana.[8-12] Salmonellosis due to consumption of raw tomato and watermelon, Shigella flexneri gastroenteritis associated onion consumption, and listeriosis associated with consumption of lettuce and cabbage also have been reported.[8]

Enteric pathogen species are becoming a major concern because it can easily transfer from the farm to the food web and cause diseases even under low ingestion doses.[13] Most coliform bacteria are not harmful, but some strains of *E. coli*, particularly the strain 0157:H7 can cause serious illness. Though vegetables can become contaminated in various ways, the use of

wastewater effluent for irrigation has been reported as the primary route. Once ingested, some *E.coli* produces Shiga toxins, which cause various syndromes such as dysentery, hemolytic anemia, hemorrhagic colitis, reduced platelet count, and thrombotic thrombocytopenic purpura.[14] According to the World Health Organization (WHO) Global Burden of Foodborne Diseases report, more than 300 million illnesses and 2000 deaths are caused by diarrheagenic *E.coli* globally.[15] Asia, the Middle East, Africa, Central and South America have the highest risk of diarrheagenic *E.coli*.[16] Among the global population, children, the elderly, and immunocompromised people are the worst affected and most vulnerable.[17] Wastewater irrigation serves as a potential route for the introduction of new pathogens into the domestic environment through the contamination of household drinking water, consumption of raw vegetables, and occupational exposure.[18-21]

Addis Ababa City gets about 90% of its fresh leafy vegetables and 61% of the overall vegetables from farmers along the Akaki Rivers which receive all waste types from multiple sources in the city including toilets and health centers.[22] Water quality reports over the last several years show that the 2 rivers are extremely polluted.[23,24] Due to the increasing demand for fresh produce, vegetable production along these rivers is mounting; however, despite high occupational exposure, farmer's awareness of both the health risks and management of wastewater hazards is very low.[25] Therefore, quantifying the fecal contamination level at different exposure stages is crucial for intervention. Moreover, the contaminants' mobility in the irrigation system can also be give useful information during intervention. *E. coli* is a good indicator of fecal contamination.[26] It also helps to determine hygiene conditions for the primary production of leafy vegetables and to verify the application of good agricultural practices (GAP)[27] as part of the Codex Alimentarius Commission's code of practice for fresh fruits and vegetables.[28] Moreover, determining the occupational and hygiene-related risk factors associated with the reuse of wastewater is the baseline information to reduce the impacts of fecal pathogens. Therefore, the objective of this research was to examine the degree of fecal contamination in wastewater irrigation systems and the potential transfer of fecal pathogens (using *E. coli* as a fecal indicator) from irrigation water to soil and crops in the irrigation system.

Materials and Methods

Description of the study area and sampling sites

This survey was carried out in nineteen urban farming sites along the 2 rivers (Big Akaki and Little Akaki Rivers) that cross Addis Ababa, the capital city of Ethiopia (Figure 1). According to the World Population Review, the population of the city by 2023 is estimated to be 546 891.[29] Only 64% of the solid waste produced is properly disposed of; about 74% of the residents use pit latrines, 7% use flush toilets, and 17% use open defecation (open defecation in the field).[30] The 2008 UN-Habitat basic indicators assessment in the city showed that 26% of the houses and the majority of slum dwellers had no toilet facilities, 33% of households shared a toilet with more than 6 households, 35% of the generated garbage/refuse was never collected, and 71% of the households did not have adequate sanitation facilities.[30] Like most Sub-Saharan African nations, polluted stream water has been used for crop production within and on the outskirts of Addis Ababa since the 1940s to produce a variety of crops for both market and home consumption.[22] More than 1240 ha of land is irrigated for vegetable production using the Akaki River only, supporting more than 1260 farming households in the city and its surroundings.[22] Almost all of these farmers use untreated wastewater and polluted rivers to irrigate the majority of the city's leafy vegetable supply.[22,25]

Sampling sites were selected based on pre-set criteria including the practice of permanent irrigation activities, using either wastewater or non-wastewater water sources but not both, and production of leafy vegetables. A total of 19 sampling sites that met the selection criteria were identified along the 2 Akaki Rivers. The 19 sampling sites included 11 sites irrigated by wastewater only (rivers receiving waste discharges directly from hosueholds), 8 sites irrigated by non-wastewater sources (non-wastewater irrigation sources include groundwater, tap water and rivers with no connection with toilet discharges). A land use record and information obtained from the farmers indicated that the non-wastewater irrigated farms had never been irrigated with wastewater. The farms are small in size and mainly used to produce vegetables for domestic use or for the nearby community supply.

Study design, data and sample collection

The data were collected using 2 study designs: cross-sectional study consisting farm and water, sanitation and hygiene (WASH) survey and microbiological analysis. The cross-sectional study was used to examine farmers exposure to wastewater pathogens. In this design, only 197 farmers using wastewater for irrigation were included, whilst microbiological analysis was carried out for both wastewater and non-wastewater irrigating farms. The latter was done for 26 farmlands (13 wastewater-irrigated farms and 13 non-wastewater-irrigated farms).

Farm and WASH (survey). The survey data were collected from 197 study population (wastewater-irrigating farmers) in the sampling sites by using cross-sectional study. The data were collected through interview and observation with the household member most engaged in farming practices. Information was gathered by using list of structured questionnaire questions focusing on factors potentially exposing the farmers to wastewater-related pathogens including personal hygiene, occupational exposure, the use of protective clothes and vegetable production, processing and consumption. Before the actual field data collection began, the tools were pilot-tested with farmers who were not included in the study by trained data collectors.

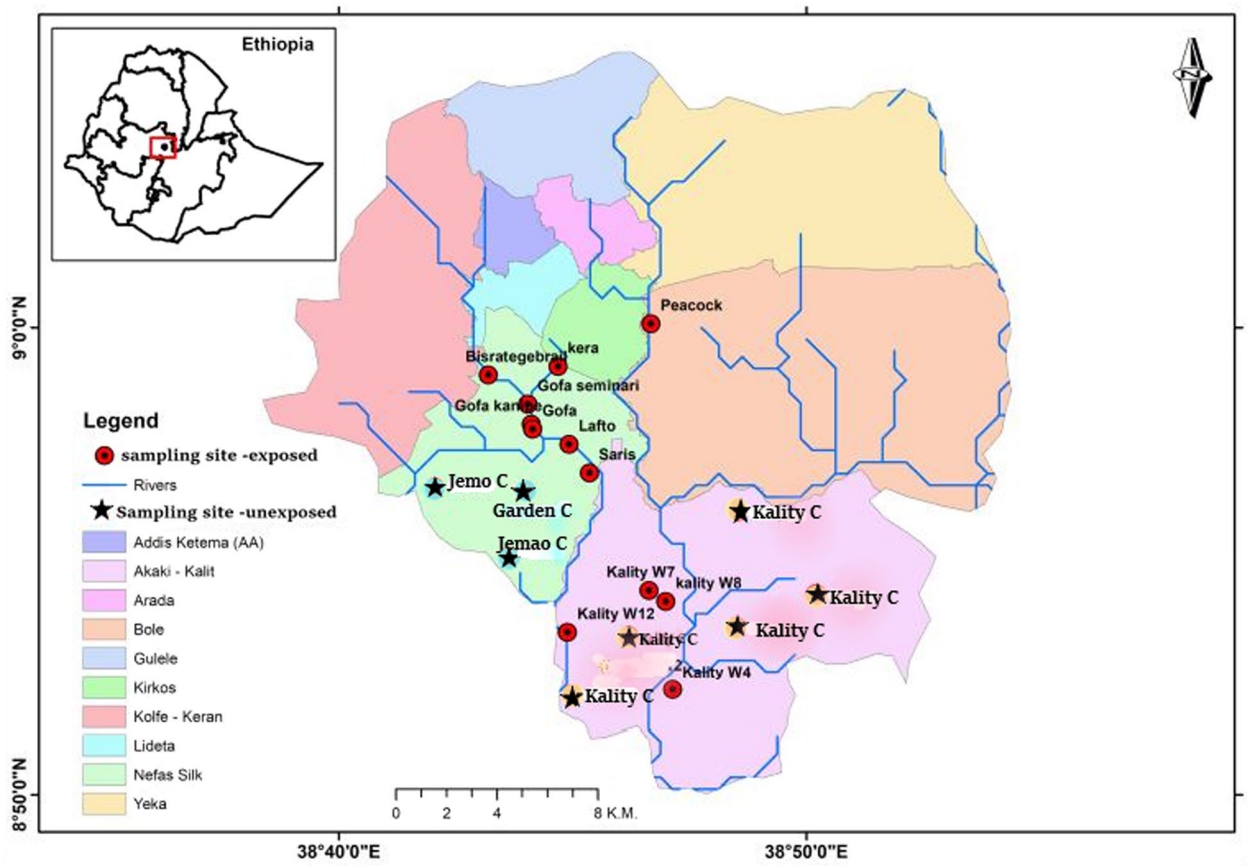

Figure 1. Study area and sampling sites.

Sample collection and microbial analysis. Sample collection and microbial analysis of irrigation water, soil and vegetables were carried out side by side with the farm and WASH surveys. During 2 vegetable growing periods (approximately February to May 2022, and October 2022 to January 2023), composite samples of irrigation water, irrigated soil (moistened soil within the irrigated land), and 2 types of leafy vegetables (lettuce and Swiss chard) were collected from the 19 sampling sites (farm lands) (Figure 1). A total sample of 208 composite samples (2×26 samples of irrigation water, 2×26 samples of soil, 2×26 samples of lettuce, and 2×26 samples of Swiss chard from 19 sampling sites) were collected during the 2 growing seasons (26 samples from 19 sites in 2 seasons).

To get representative composite samples, irrigation water, soil and vegetable samples were taken from 3 points within each plot. 250 ml of composite irrigation water samples were collected with sterilized sampling bottles; about 100 g of composite healthy and edible lettuce and Swiss chard samples, which did not have direct contact with the soil and 100 g of irrigated composite soil samples were collected from each sampling site in sterilized plastic bags. All the samples were properly labeled, kept in an icebox, and conveyed to the laboratory within 4 hours of collection for further processing. The analyses of the samples were done at Addis Ababa Water and Sewerage Authority (AAWSA) central laboratory and the Kotebe University of Education microbiology laboratory. All the samples were processed by membrane filtration.

Irrigation water samples were subjected to serial dilution (10^{-1}, 10^{-2}, and 10^{-3}), and then for the enumeration of *E. coli*, 100 mL of diluted sample was membrane filtered via 0.45 µm pore size and 37 mm diameter membrane filters in duplicates aided with a vacuum pump.[31] The *E.coli* enumeration in the irrigation water was done following standard methods of membrane filtration counting.[32] 25 grams of vegetable samples were taken, rinsed with 225 ml of sterilized water, and shaken to wash the bacteria from the vegetables surface and get into the solution. Similarly, 25 g of soil samples were dissolved in 225 ml of sterilized water, and shaken to break up the soils, and get into the solution. Like irrigation water, the vegetable-washed and soil-dissolved water samples (the homogenate supernatant) were also subjected to serial dilution (10^{-1}, 10^{-2}, and 10^{-3}). Enumeration of *E. coli* on vegetable samples was also done following standard methods of membrane filtration technique.[33] Sample filtration was done similarly to the water samples.

All the membranes of irrigation water samples, vegetable-washed samples, and soil-washed-samples were incubated for 24 hours at 44.5°C in aluminum petri dishes (47 mm with pad) that have lauryl sulfate broth for *E.coli* growth on well-wetted pad. For each serial dilution across the 4 sample types, Petri dishes containing between 20 and 60 colonies were selected for enumeration.[34] For vegetable and soil samples, the number of

bacterial colonies counted was reported as CFU/g after calculating with the dilution factors; whilst for the irrigation water samples, the count was reported as CFU/100 ml of a water sample. In cases where more than one replicates had ideal colony counts, the results were converted to reporting units, and then averaged results were taken. Petridishes containing colony too few to count (TFTC) and too numerous too count (TNTC) were not considered.

Data analysis

Bacterial counts were $\log_{10}$ transformed before performing statistical analyses to minimize skewness and to ease the interpretation. The analysis was carried out by using STATA ver.14 (Stata Corp, College Station, TX) and Minitab ver. 16. Descriptive statistics were applied to summarize the basic information about variables in the dataset. The mean microbial count variations among the 3 components of the irrigation system were determined by checking for significant differences. The differences in *E.coli* concentration between the irrigation water sources and the WHO and other irrigation water quality standards were analyzed by using one-sample t-test. Two-sample independent t-test was also employed to examine the differences in *E. coli* load on the leafy vegetables between different irrigation water sources. The association between the *E.coli* load in the irrigation water and irrigated soil with the *E.coli* load on vegetables was evaluated by using a negative binomial regression model.

Factor analysis was used to simplify several exposure variables into few dimensions/categories of variables. This made the descriptive analysis easy and evaluate the effect on the outcome variables.

Result and Discussion

E.coli occurrence in the irrigation water and irrigated soil

The irrigation water samples taken from the 2 polluted rivers (Big Akaki and Little Akaki Rivers), and all the soil samples taken from wastewater-irrigated farms were positive for *E.coli*. However, during the 2 sampling phases, 12% of the non-wastewater irrigation water samples and 23% its irrigated soil samples were *E.coli* negative. Table 1 shows the descriptive statistics of the *E.coli* load of the irrigation water and soil. The concentration of *E. coli* in the irrigation water and irrigated soil varied considerably.

The mean *E.coli* load in wastewater, non-wastewater sources, and wastewater-irrigated soil were $1.1^{6} \pm 5.45^{3}$ CFU/100 ml, $2.23^{2} \pm 1.29^{2}$ CFU/100 ml, and $3.6^{2} \pm 1.6^{2}$ CFU/g respectively. The occurrence of *E. coli* in the water samples has been taken as an indicator of recent fecal pollution of the water body.[35] *E.coli* is normally found in the intestine of vertebrates, including humans, and is defecated into the surrounding environment through feces or wastewater effluent. Thus, *E. coli* is taken as the best indicator of the bacteriological quality of water.[36] Therefore, the occurrence of *E.coli* in the irrigation water and irrigated soil, in this study, is an indication of fresh fecal contamination in irrigation water and irrigated soil. The variability of *E.coli* load between different measurements and locations in the waterway may be due to the source of the contaminants, the timing of disposal, and survival of the contaminants in the environment, and other factors including ambient environmental conditions, availability of nutrients and energy sources, and water quality.[35] Moreover, the changes in the toilet discharge time from the

Table 1. Descriptive statistics of the *E.coli* load in the irrigation water sources (CFU/100 ml) and irrigated soil (CFU/g).

DESCRIPTIVE STATISTICS	WASTEWATER	WASTEWATER-IRRIGATED SOIL	NON-WASTEWATER WATER	NON-WASTEWATER-IRRIGATED SOIL
Mean	1.1^{6}	3.64^{2}	2.23^{2}	1.32^{2}
Standard dev.	5.45^{3}	1.6^{2}	1.3^{2}	87.1
Variance	2.97^{7}	2.48^{4}	1.65^{4}	7.58^{3}
Min	5.5^{5}	1.4^{2}	50	0
Q1	6.3^{5}	2.4^{2}	1.5^{2}	67.5
Median	8.5^{5}	3.2^{2}	1.5^{2}	1.35^{2}
Q3	1.7^{6}	5^{2}	3.3^{2}	2.03^{2}
Max	2^{6}	6.8^{2}	4.5^{2}	3.2^{2}
Range	1.4^{6}	5.4^{2}	4.00	3.2^{2}
IQR	1.1^{6}	2.5^{2}	1.75	1.4^{2}
skewness	0.74	0.49	0.78	−0.45
Kurtosis	−1.17	−0.38	0.62	0.18

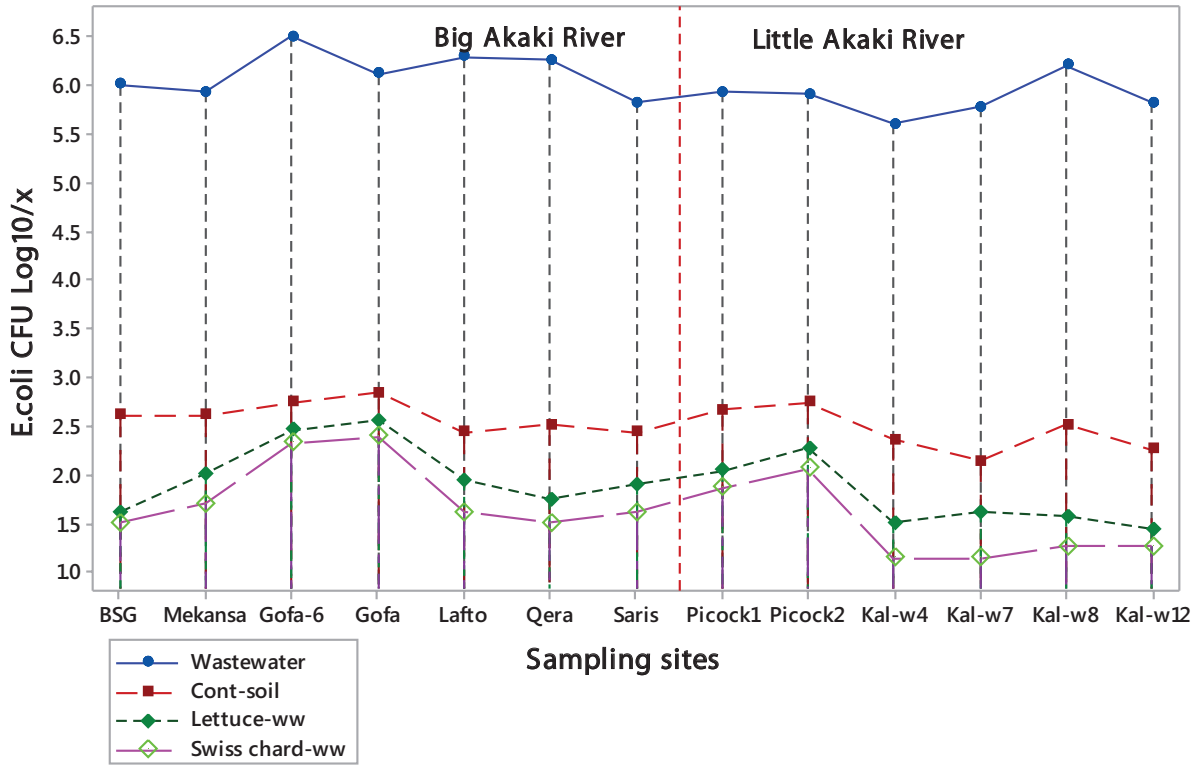

Figure 2. Comparative spatial variations of *E.coli* load in the leafy vegetables, irrigated soil and wastewater.
x = gram for vegetables and soil, and 100 ml for wastewater, BSG = Bisrategebrael, Mekanisa = Gofa camp, Gofa-6 = Gofa seminari, Kal = Kality; cont-soi = contaminated soil, Lettuce ww = Letuce produced by using wastewater, Swiss chard ww = Swish chard produced by using wastewater.

resident houses built along the river and sampling time differences can affect the *E.coli* variability in the contaminated river. Discharges from health centers including hospitals and industries can also cause variability of the *E.coli* population in the rivers.[37] The most important source in the urban environment includes open defecation, connecting septic tanks and toilets with the rivers, and damage to sewerage systems.[30] The differences in *E.coli* in the irrigation water and irrigated soil may be due to several factors including differences in the survival of *E.coli* in the soil and water environment. This is because the 2 environments offer different conditions to the microbial community such as pH, temperature, availability of nutrients and energy sources, presence of other microorganisms, and ability to form biofilm in the secondary environments.[35] *E.coli* can survive in water for 4 to 12 weeks,[38] whereas in the soil, it can live for more than 200 days depending on the type of strain and different environmental factors.[39]

Spatial variabilities of fecal contamination in the irrigation system

Spatial fecal contamination variability in the irrigation system along the Akaki Rivers was estimated as *E.coli* CFU load in the irrigation water, irrigated soil, and vegetables (Figure 2). In the wastewater, the maximum *E.coli* load was recorded around the mid-length of Little Akaki River, between upstream and downstream (Gofa Kamp = 6.5 $\log_{10}$/100ml, Lafto = 6.29$\log_{10}$/100ml), and Qera = 6.25$\log_{10}$) and downstream of Big Akaki River (Kality *w8* = 6.20 *E.coli* $\log_{10}$/100 ml) (Figure 1). The minimum *E.coli* load in wastewater was recorded at the Kality w7 site (Figure 1), downstream of Little Akaki River (Figure 1) (5.778$\log_{10}$/100ml), showing that the minimum microbial load in the 2 rivers was found at the farming plots located downstream of the 2 rivers. Similarly, the *E.coli* load in the irrigated soil and the leafy vegetables varied significantly across the sampling sites. These minimum *E.coli* loads in the irrigated soil and the leafy vegetables were recorded in sites where minimum *E.coli* loads in the wastewater were found. The maximum *E.coli* loads were also associated with the maximum values recorded in the irrigation water.

Wastewater is a favorable habitat for microbial growth, however, factors such as variabilities in nutrients and oxygen concentration, and the discharge of toxic substances from the source can affect the microbial population.[40] The *E. coli* count in a given environment is influenced not only by the sources but also by the prevailing physical, biological, and chemical factors in the environment.[41-43] The maximum *E.coli* count at the 3 sites along the Little Akaki River may be due to the direct river-toilet connection of the crowded inhabitants with poor sanitation facilities close to the river sites. Moreover, these sites are relatively flat so the slow velocity of the river may give relatively better survival opportunities for the *E.coli*.[44] Reports show that some specific strains of *E. coli* can live for months or years, and possibly grow, in extra-intestinal environments.[35,45]

Table 2. Microbial quality differences between the different types irrigation water sources (n = 26).

MEAN LOG10 *E.COLI*	WASTEWATER GROUPS		WASTEWATER VS NON-WASTEWATER		WHO VS IRRIGATION WATER	
CFU /100 ml	Big Akaka	Little Akaki	Wastewater	Non-wastewater	Non-wastewater	Wastewater
	5.81	5.82	5.82	2.3	2.3	5.82
95% CI	5.7-3.9	5.7-4.0	5.7-5.9	2.1-2.4	2.1-2.4	6.7-5.9
t-test	−0.1		18.0		−9.7	19.6
P-value	>.05		$P < .001$		<.001	<.001

The *E.coli* load in the irrigation system in an order of magnitude from lowest to highest was Swiss chard, lettuce, irrigated soil, and wastewater. But, it should be understood that the irrigation water is the ultimate source of contamination to the irrigated soil and the vegetables.

The graph clearly shows the simultaneous fluctuations of the *E.coli* load in the irrigation systems at the same sampling sites. This implies that the various loads of *E.coli* in the irrigation water cascade down to the system, and thus the corresponding *E.coli* load in the irrigated soil and the vegetables were high or low depending on the load in the wastewater. Both the maximum and minimum *E.coli* counts in the wastewater represents to maximum and minimum *E.coli* counts in the soil and the vegetables respectively. This is an indication that the microbial mobility in the irrigation system is high and therefore vegetables produced from most polluted river segments usually have more fecal contaminants and thus, have poor microbial quality.

Microbial quality of the irrigation water and soil

The microbial quality of the wastewater used for irrigation was compared to the non-wastewater irrigation water sources and to the international irrigation water standards using a t-test (Table 2). There were significant differences between the microbial quality of the wastewater used for irrigation and the non-wastewater irrigation water sources, $t(12) = 18.0$, $P < .001$).

The presence of *E. coli* in the groundwater, the river water, or the tap water indicates contamination of water with fresh fecal matter that may contain other harmful or disease-causing microorganisms including bacteria, viruses, and parasites.[35] Similarly, the microbial quality of both the contaminated and non-wastewater irrigation water sources significantly differ from the WHO standards, t (12) = −9.7, $P < .001$) and t (12) = 19.6, $P < .001$ respectively (Table 2).

Figure 3 shows the microbial quality of irrigation water and irrigated soil by irrigation water sources compared to WHO and other irrigation water quality standards. Irrigation water quality guideline and standards for wastewater reuse in agriculture vary considerably from country to country.[46] The 2006 WHO water guideline defines health-based targets regarding wastewater reuse, indicating that $<6\log_{10}$ disability-adjusted life years should be induced as one of the 4 components of the health-based targets.[47] According to the report, this is equivalent to $<3\log_{10}$ – $4\log_{10}$ *E.coli*/100 ml wastewater.

For restricted irrigation, less than $5\log_{10}$ *E.coli* CFU/100 ml is acceptable, whereas for unrestricted irrigation of crops eaten raw, the *E.coli* CFU/100 ml should be $3\log_{10}$ is acceptable.[47] The irrigation water sources that have a direct trace on the edible part of a crop should not have more than $2.37\log_{10}$ CFU generic *E. coli*/100 mL for any single sample with a geometric mean (n = 5) of $\leqslant 2.1\log_{10}$ CFU *E.coli*/100 mL of water.[48] More recently, the US Food and Drug Administration (USFDA) reviewed the maximum limit of the irrigation water quality criteria to a threshold value (STV) (ie, a value that should not be exceeded by more than 10% of the samples taken) $\leqslant 2.613\log_{10}$ CFU of generic *E. coli*/100 mL of water.[49] All of the wastewater samples did not meet the WHO standards for unrestricted irrigation water ($3\log^{10}$) and the strictest irrigation water standards ($2.37\log_{10}$). Although all of the non-wastewater irrigation water sources meet the WHO standards for unrestricted irrigation, 84.6% did not met the standard for irrigation used for edible crops which has direct contact with the irrigation water.

In this study, the *E.coli* load in all wastewater samples was beyond the WHO's standard for crops whose edible part can directly contact the irrigation water. This indicates that the irrigation water is not acceptable for the production of crops like lettuce and Swiss chard because these crops are low-growing crops and soil and are normally eaten raw. Likewise, the *E.coli* count in the present study was also much higher than the USFDA standard. However, the quality of the non-wastewater irrigation water sources is below the standards set by the WHO ($4\log_{10}$ CFU/100 ml) and USFDA ($2.65\log_{10}$/100ml) for the use of wastewater to grow crops that are normally eaten raw. The most common leafy vegetables produced in the study area, apart from lettuce and Swiss chard, include Ethiopian kale, cabbage, and cauliflower.

Fecal contamination of leafy vegetables growing on wastewater irrigation

The *E.coli* load on lettuce (CFU/g) varies between 1.43 ± 1.1 $\log_{10}$ (27 ± 12.7) at the Kality site (downstream) and 2.55 ± 0.81 $\log_{10}$ ($3.56^2 \pm 6.4$) at the Gofa sites; and on

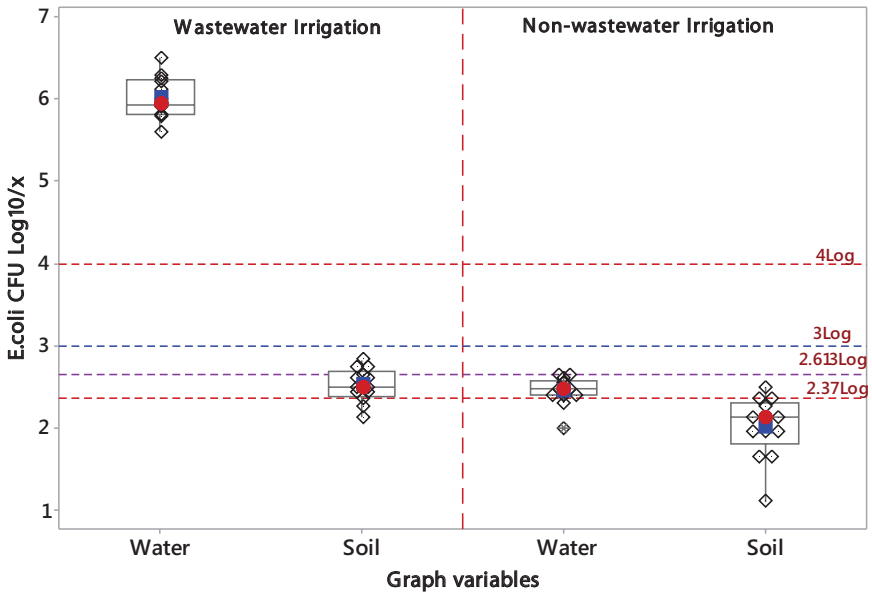

Figure 3. Irrigation water and soil quality by the type of water irrigation water sources.
The 4 lines are different standards for the irrigation water only. Line = 2.37Log is irrigation water standard for crops whose edible part directly contacts the irrigation water. Lines = 2.613log$_{10}$ is the US FDA Statistical Threshold Value of *E.coli* load in irrigation water, Line = 3Log$_{10}$ is the WHO limit of *E.coli* load for unrestricted drip irrigation for low-growing crops. Line = 4Log$_{10}$ is the WHO limit of *E.coli* load for unrestricted irrigation water, for leafy crops normally eaten raw. X=100ml for the irrigation water, and g for the irrigated soil. Diamond symbols = individual data, box = 95% CI for median interval, Red circle = median, and Blue rectangle symbol = mean, horizontal line = median line, the vertical lines are upper and lower whiskers.

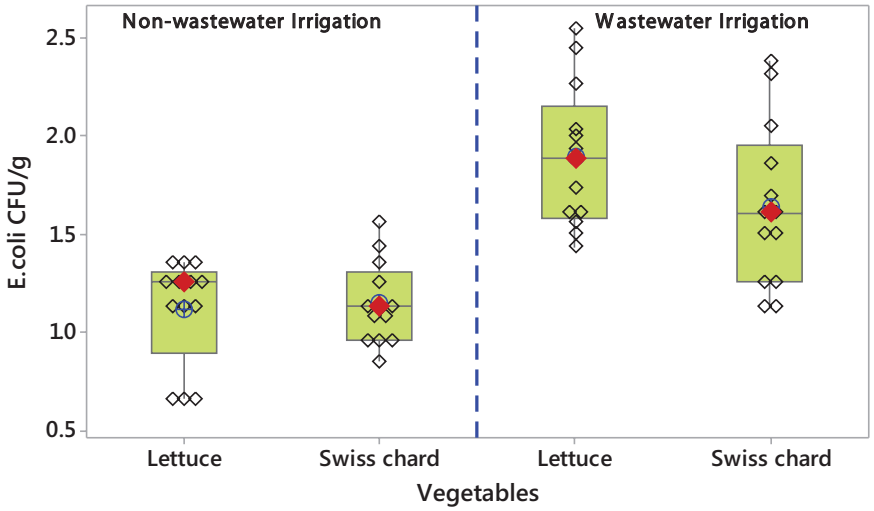

Figure 4. *E.coli* concentration in the vegetables grown by using wastewater and surface and ground water.
Key: red circle = Median value, blue crossed-circle = mean value.

Swiss chard, the count varies between 1.26 ± 1.1 $\log_{10}$ (18 ± 12.7) at Kality sites and 2.39 ± 2.01 $\log_{10}$ ($2.43^2 \pm 1.02^2$) at Gofa sites. The maximum *E.coli* loads of the 2 vegetables were recorded at Gofa sites and the minimum at Kality sites (Figure 1). The wide variations between separate *E.coli* counts (large standard deviations) and differences in the *E.coli* count of the different sampling sites of the farmland along the river bank may be because of the high dynamicity of the *E.coli* population and thus fecal contamination at source as well.

Figure 4 shows the *E.coli* load on lettuce and Swiss chard produced from different irrigation water sources. For the farming plots using wastewater, the mean *E.coli* load on lettuce (1.89 ± 0.36 $\log_{10}$/g) was higher than the mean *E.coli* load on Swiss chard (1.64 ± 0.42 $\log_{10}$/g). But, in non-wastewater farming plots, the mean *E.coli* load for lettuce (1.11 ± 0.27 log10/g) all counts were low compared to the mean *E.coli* load of Swiss chard (1.14 ± 0.2 1$\log_{10}$/g). This may indicate the variabilities of the vegetables in providing favorable conditions under different fecal contamination levels. Researchers show that chemical factors including the availability and type of nutrients can affect the survival and growth of pathogens on vegetables.[50]

There was no significant differences in the *E.coli* counts between lettuce and Swiss chard on both contaminated rivers, $t(11) = 1.63$, $P = .12$, despite the mean *E.coli* load of both lettuce

Table 3. Negative binomial regression analysis of the effect of *E.coli* load of the wastewater and irrigated soil on the microbial quality of lettuce and Swiss chard.

	IRR[a]	COEFF.	Z	P>/Z/	95% CI
E.coli CFU/g lettuce[b]					
▪ Wastewater	1.000001	0.0000009	0.01	.098	–1.00017, 0.000172
▪ Irrigated soil	1.004599	0.004588	4.48	.000	0.0019478, 0.007229
- const	15.51584	2.4719	8.72	.000	–3.21361, 3.5514
E.coli CFU/g Swiss chard[c]					
▪ Wastewater	1.000016	0.000016	0.24	.08	–0.0001517, 0.0001829
▪ Irrigated soil	1.005507	0.005492	5.38	.000	0.0028638, 0.0081194
- const	5.611523	1.72482	5.62	.000	0.9345375, 2.515107

[a]Incidence rate ratio.
[b]model: $x^2(2)$ lettuce = 92.97, $P < .001$.
[c]model: $x^2(2)$ Swiss chard = 54.33, $P < .001$.

and Swiss chard produced by using Little Akaki River being higher than the *E.coli* load of lettuce and Swiss chard produced by using Big Akaki River. Nevertheless, vegetables produced by using contaminated irrigation water contain significantly higher concentrations of *E.coli* than vegetables produced by using non-wastewater irrigation water sources, $t(24) = 6.2$, $P < .001$ for lettuce, and $t(24) = 3.8$, $P < .001$ for Swiss chard respectively. Leafy vegetables are prone to microbial contamination through several routes such as direct contact between irrigation water and the edible portion of the crop and splash of irrigation water from the soil onto the leaves at different stages of the production process.[51,52] Studies in other Sub-Saharan cities also reported likewise high levels of fecal contamination of lettuce produced with wastewater.[20] Another study in Addis Ababa pointed out high levels of fecal coliforms ranging from 3.46 to 5.03 $\log_{10}$MPN/g on fresh lettuce produce from Akaki River.[53]

The sources of *E.coli* on the vegetables can be through contact with the contaminated water and the soil or cross-contamination by humans during farm activities or other animals. The levels of fecal contamination of vegetables depend on the growing conditions as well as the exposure and contact with soil, manure, or irrigation water.[54] In the present study, the irrigation water and soil were unacceptable for growing all types of vegetables particularly for low-growing leafy vegetables, both the irrigation water and the soil were not appropriate. It should be understood that for both the soil and vegetables, the ultimate source of contaminants comes from the wastewater. The magnitude and variability of *E.coli* in the soil and vegetables depend on the *E.coli* load and variability in the wastewater.

The effect of E.coli load in wastewater and soil on the quality of vegetables

Due to the inflated variance of the *E.coli* count and a small number of zero values, a negative binomial regression model was used to evaluate the relationship between wastewater and irrigated soil with the vegetables (Table 3). The overall model fit was significant [model: $x^2(2) = 92.97$, $P < .001$ for lettuce, and model: $x^2(2) = 54.33$, $P < .001$, for Swiss chard, showing that the overall relationship between the *E.coli* on the vegetables and *E.coli* load in the irrigation water and soil was well explained. The analysis indicated that the *E.coli* load of both vegetables showed a significant positive relationship with the *E.coli* of the wastewater and the irrigated soil. For instance, with a one unit CFU increase in the irrigated soil, the $\log_{10}$ count of the *E.coli* on the lettuce is expected to increase by [0.005 (b = 0.005, $P < .001$, $x^2(2) = 92.97$, 99% CI (0.002, 0.007)], given the other predictor variables in the model are held constant. For every *E.coli* increase in the soil, the incidence on the vegetable increases by 4.4%. Similarly, the *E.coli* count in the irrigated soil also showed a strong association with the *E.coli* count on the Swiss chard ($b = 0.0055$, $P < .001$). Although it is not significant, the irrigation water also influenced the levels of *E.coli* in the vegetables. For each one $\log^{10}$ unit increase in the *E.coli* count in the wastewater, the $\log_{10}$ count of *E.coli* CFU in the Swiss chard is expected to increase by approximately 0.00002. In other words, for each $\log^{10}$ unit increase of *E.coli* in the wastewater, in the Swiss chard, it increases by 0.4% [$b = 0.0002$, $x^2(2) = 54.3$, $P = .08$, 99% CI (–0.00015, 0.00018)]. The morphology and structure of leaves may also affect the ability to retain water on their surface so that the survival and population size of the pathogens can vary accordingly. For instance, wrinkled, rough surface and folded leaves can retain more water and soil than smooth and unfolded varities.[55] Moreover, the binding kinetics of different pathogens to the surfaces of the leaves (adherence) may also contribute the daynamics of pathogens on the leaves surface.[56,57]

The contamination of vegetables grown in soil irrigated with fecal-contaminated water will largely depend on the survival capabilities of the pathogens in the wastewater, soil, and vegetables.[58,59] Several reviews highlighted the effect of microbial quality

of irrigation water on the pathogenic populations on vegetable products have been published.[60,61] Several research findings also linked vegetable contamination to contact with soil,[62,63] others reported that the irrigation water is more determinant.[64,65] Where leaves contamination takes place, depending the type of the pathogens present and their pathogenesity, pathogen tenacity and survival may induce a risk to consumers' health.[66] The longer the pathogens survive in the wastewater or soil, the greater the potential they have to become in contact with individuals and the environment.[67] An increased contaminant persistence and survival in the wastewater and soil increases the chance of spreading the contaminants into the community and household environment. Thus, there will be an increased risk of contamination and infections to the farmer, their families, and the consumers.

Potential risks of fecal contamination in the irrigation system

The proportion and factor analysis of the potential risk factors of fecal contamination and mitigating measures data was collected through observation and interviewing presented (Table 4). The levels of farmers' exposure to fecal contamination were high. The greatest risk identified were as follows: approximately 90% hand contamination, 83% eating raw vegetables, 73% using their working at home, 68% using irrigation water for body washing and 68% walking through the irrigation water were the highest risk factors. On the other hand, farmers' practice toward mitigating factors were found to be very poor, ranging from only 10% of them washing their hands with soap before eating to a maximum of 48% practicing onsite hand washing.

Factor analysis (multivariate analysis) was carried out for both exposure (behaviors that put farmers at risk) and mitigating variables (behaviors that protect farmers from the risk of contamination or infection) separately. The analysis indicated a strong correlation between the variables and the factors (factor in factor analysis are latent variables created as result of a set of observed variables that have similar response patterns giving different levels as factor 1, factor 2 etc). Among the exposure variables, washing vegetables with irrigation water (0.788) and consuming raw vegetables (0.827) have large loadings on factor 1 indicating that the exposure variables are strongly correlated to the factor. Hand contamination by soil and water (0.761) and using working clothes at home (0.727) have also large loadings on factor 2 indicating a strong correlations between the variables and the factor. Washing body with irrigation water (0.902) and touching body with contaminated hands (0.876) have also large positive loadings on factor 3 and 4 respectively indicating strong correlation between the exposing

Table 4. Descriptive Statistics of exposure variables to fecal contaminants for farmers working on wastewater irrigation (n = 197).

VARIABLES	"YES" RESPONSE NUMBER (%)	FATOR LOADING (F*)
Exposing variables		
Wash vegetables with irrigation water	126 (64)	0.788 (F1)
Eating raw vegetables	163 (83)	0.827 (F1)
Hand contamination by soil and water	178 (90)	0.761 (F2)
Cloth contamination with soil and water	107 (54)	0.430 (F2)
Using working clothes at home	144 (73)	0.727 (F2)
Wash body with irrigation water	133 (68)	0.902 (F3)
Touch body with contaminated hands	122 (62)	0.876 (F4)
Walking through irrigation water	134 (68)	0.368 (F4)
% Variance		0.644
Mitigating variables		
Wearing protective equipment	64 (33)	0.905 (F1)
Taking a bath after irrigation work	68 (35)	0.876 (F1)
Onsite hand washing	95 (48)	0.509 (F2)
Always washing feet and shoes after work	38 (19)	0.728 (F2)
Regular hand washing after farm work	40 (20)	0.679 (F3)
Washing hands with soap before eating	22 (11)	0.766 (F3)
% variance		0.601

F = F1*, F2 etc .. referes to factor1, factor 2.

variables and the factor. Together, all the 4 factors explained 0.644 or 64.4% of the variation in the data. Therefore, washing vegetables with irrigation water, consuming raw vegetables, hand contamination by soil and water, using working clothes at home, washing body with irrigation water and touching body with contaminated hands are identified as the major exposure variables to the large E.coli loads in the wastewater, irrigated and vegetables.

Among the mitigating variables, wearing protective equipment (0.905) and taking a bath after farm work (0.876) have large positive loadings on factor 1 indicating strong correlation between mitigating variables and factor 1. Washing hands with soap before eating (0.766) and regular hand washing after farm work (0.679) have also large loadings on factor 3. Together, all the 3 factors explained 0.601 or 60.1% of the variation in the data. Therefore, although wearing protective equipment, taking a bath after farm work, washing hands with soap before eating and regular hand washing after farm work were poorly practiced by wastewater-irrigating farmers, they are confirmed to be key mitigating variables.

While an average 70% of the farmers are exposed to fecal contaminants as defined by "no hand washing and non changes of clothes," an average 28% of the farmers implement mitigating factors such as wearing protective clothes, washing hands and feet (Table 4). The big difference between practicing exposure and protective behaviors indicate an increased potential of infection. The elevated levels of *E coli* quantified on the leafy vegetables and the absence of preventative measures such as glove use, hand washing, changing clothes before arriving in the house, there is potential for high levels of exposure to these workers. The primary drivers appear to be wash body with irrigation water, touch body with contaminated hands, eating raw vegetables, wearing protective equipment, and taking a bath after irrigation work (Table 4). Therefore, high exposure rates combined with poor hygiene behavior and low protective equipment use would actually increase the infection rate and suggest the need for an intervention. All major farm activities like forking and weeding, which are causes of hand and clothes contamination, were regarded as risk activities found to expose farmers to fecal contamination.[36] Farmers may ingest fecal contaminants when they accidentally touch their mouth, nose, or eyes with contaminated hands or clothes during farm work. Reports show that all exposed populations take in at least small quantities of soil attached to the fingers because of hand-to-mouth movement.[36,68]

Conclusion

The *E.coli* load in the wastewater compared to both WHO and FSMA FDA standards, is not acceptable for all types of vegetable production, particularly for the production of leafy, low-growing vegetables. The high level of *E.coli* in the wastewater contaminates the soil, which may give a conducive environment for the pathogens to survive and grow. In the presence of high levels of *E.coli* in the irrigation water, soil, and vegetables, and high level of exposure in the absence of using protective equipment and poor hygiene behavior, the risk of contamination for the farming households and the whole community will be high and put communities at risk of E.coli infection. Therefore, interventions such as on-farm wastewater treatment to reduce pathogen load in the wastewater and improved hygiene practice to interrupt the transmission are recommendable. To reduce the potential hazards of wastewater-related infections, relevant public health education interventions can be used to help inform the communities at greatest risk. Public health interventions are also required to inform and educate vulnerable individuals on food preparation to reduce the chances of infection for the foreseeable future. Future studies should focus on measuring actual symptomatic disease incidence in Akaki River farming populations to quantify the risk of enteric disease due to exposure to wastewater or measure levels of *E coli* on farmworkers hands and in their households as an exposure stepping-stone.

Acknowledgements

We would like to thank Water and Land Resource Center of Addis Ababa University for their timely resource support. We are very grateful to Water Security and Sustainable Development Hub, The UK Research and Innovation's Global Challenges Research Fund (GCRF). We are also thankful to Addis Ababa Water and Swerage Authority for allowing us to work in their standard laboratory. We also appreciate Wagtech-Ethiopia for the laboratory in put supply and timely material support.

Availability of Data and Materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethical Clearane

This research was carried out in accordance with the National Research Ethics Review Guidelines Fifth edition. We obtained ethical approval and clearance from National Research Ethics Review Committee, under Ethiopian Ministry of Education.

REFERENCES

1. Khalid S, Shahid M, Bibi I, Sarwar T, Shah AH, Niazi NK. A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-income countries. *Int J Environ Res Public Health*. 2018;15:895.
2. Sato T, Qadir M, Yamamoto S, Endo T, Zahoor A. Global, regional, and country level need for data on wastewater generation, treatment, and use. *Agric Water Manag*. 2013;130:1-13.
3. Drechsel P, Graefe S, Sonou M, Cofie O. *Informal irrigation in West Africa: An overview. IWMI Research Report No. 102*. Colombo, Sri Lanka; 2006.
4. Drechsel P, Bahri A, Raschid-Sally L, Redwood M. *Wastewater Irrigation and Health: Assessing and Mitigating Risk in Low-Income Countries*. IWMI; 2010.
5. Jhansi SC, Mishra SK. Wastewater treatment and reuse: sustainability options. *Consilience*. 2013;10:1-15.
6. Pratap B, Kumar S, Purchase D, Bharagava RN, Dutta V. Practice of wastewater irrigation and its impacts on human health and environment: a state of the art. *Int J Environ Sci Technol*. 2023;20:2181-2196.

7. Iwu CD, du Plessis E, Korsten L, Okoh AI. Prevalence of *E. coli* O157: H7 strains in irrigation water and agricultural soil in two district municipalities in South Africa. *Int J Environ Stud*. 2021;78:474-483.
8. Beuchat LR. Pathogenic microorganisms associated with fresh produce. *Journal of food protection*. 1996;59(2):204-216.
9. Kozak GK, MacDonald D, Landry L, Farber JM. Foodborne outbreaks in Canada linked to produce: 2001 through 2009. *J Food Prot*. 2013;76:173-183.
10. Wu Y, Wen J, Ma Y, Ma X, Chen Y. Epidemiology of foodborne disease outbreaks caused by Vibrio parahaemolyticus, China, 2003–2008. *Food Control*. 2014;46:197-202.
11. Udo S, Andy I, Umo A, Ekpo M. Potential human pathogens (bacteria) and their antibiogram in Ready–to–eat salads sold in Calabar, South-South, Nigeria. *Internet J Trop Med*. 2009;5:1.
12. Nasser AM. Transmission of Cryptosporidium by Fresh Vegetables. *J Food Prot*. 2022;85:1737-1744.
13. Burgess CM, Gianotti A, Gruzdev N, et al. The response of foodborne pathogens to osmotic and desiccation stresses in the food chain. *Int J Food Microbiol*. 2016;221:37-53.
14. Lupindu AM. Epidemiology of Shiga toxin-producing Escherichia coli O157:H7 in Africa in review. *South Afr J Infect Dis*. 2018;33:24-30.
15. Troeger C, Blacker BF, Khalil IA, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis*. 2018;18:1211-1228.
16. Havelaar AH, Kirk MD, Torgerson PR, et al. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS Med*. 2015;12:e1001923.
17. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet*. 2013;382:209-222.
18. Falkenberg T, Saxena D. Impact of wastewater-irrigated urban agriculture on diarrhea incidence in Ahmedabad, India. *Ind J Commun Med*. 2018;43: 102-106.
19. Andoh LA. *Helminth Contamination of Lettuce and Associated Risk Factors at Production Sites, Markets, and Street Food Vendor Sites in Urban and Peri-Urban Kumasi*. Kwame Nkrumah University of Science and Technology; 2006.
20. Adegoke AA, Amoah ID, Stenström TA, Verbyla ME, Mihelcic JR. Epidemiological evidence and health risks associated with agricultural reuse of partially treated and untreated wastewater: A Review. *Public Health Front*. 2018;6:337-337.
21. Nadabo C, Ramyil SC, Bello CS, et al. Parasitic contamination of commonly consumed and locally cultivated leafy vegetables in Jos, north-central Nigeria. *J Human Environ Health Prom*. 2022;8:1-9.
22. Alebel A, Tesema C, Temesgen B, Gebrie A, Petrucka P, Kibret GD. Prevalence and determinants of diarrhea among under-five children in Ethiopia: a systematic review and meta-analysis. *PLoS One*. 2018;13:e0199684.
23. Gessew GT, Desta AF, Adamu E. High burden of multidrug resistant bacteria detected in Little Akaki River. *Comp Immunol Microbiol Infect Dis*. 2022;80:101723.
24. Mengesha SD, Asfaw YB, Kidane AW, et al. Microbial risk assessment and health concern of vegetables irrigated with Akaki River in Addis Ababa, Ethiopia. *Sci Afr*. 2023;19:e01541.
25. Woldetsadik D, Drechsel P, Keraita B, Itanna F, Gebrekidan H. Farmers' perceptions on irrigation water contamination, health risks and risk management measures in prominent wastewater-irrigated vegetable farming sites of Addis Ababa, Ethiopia. *Environ Syst Decis*. 2018;38:52-64.
26. Dufour A. *Escherichia coli: The Fecal Coliform*. ASTM International; 1977.
27. Allende A, Monaghan J. Irrigation water quality for leafy crops: a perspective of risks and potential solutions. *Int J Environ Res Public Health*. 2015;12: 7457-7477.
28. Alimentarius C, Cometti NN, Matias GCS, Zonta E. Code of hygienic practice for fresh fruits and vegetables. *Cometti, NN, Matias, GCS, Zonta, E*; 2003.
29. Review Wp. Addis Ababa Population Data. Accessed 22 July 2022. https://population.un.org/wpp/FAQs/
30. UN-ECA. *The State of African Cities 2008: A Framework for Addressing Urban Challenges in Africa*. UN-HABITAT; 2008.
31. Adefisoye MA, Okoh AI. Identification and antimicrobial resistance prevalence of pathogenic Escherichia coli strains from treated wastewater effluents in Eastern Cape, South Africa. *Microbiologyopen*. 2016;5:143-151.
32. Blumenthal UJ, Mara DD, Peasey A, Ruiz-Palacios G, Stott R. Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. *Bull World Health Organ*. 2000; 78:1104-1116.
33. Refai M. *Manuals of food quality control. 4: Microbiological analysis*; 1979.
34. APHA. 9222 Membrane filter technique for members of the coliform group. *Standard Methods For the Examination of Water and Wastewater*; 2018:27
35. Jang J, Hur HG, Sadowsky MJ, Byappanahalli MN, Yan T, Ishii S. Environmental*Escherichia coli*: ecology and public health implications-a review. *J Appl Microbiol*. 2017;123:570-581.
36. Antwi-Agyei P, Biran A, Peasey A, Bruce J, Ensink J. A faecal exposure assessment of farm workers in Accra, Ghana: a cross sectional study. *BMC Public Health*. 2016;16:1-13.
37. Kilunga PI, Kayembe JM, Laffite A, et al. The impact of hospital and urban wastewaters on the bacteriological contamination of the water resources in Kinshasa, Democratic Republic of Congo. *J Environ Sci Health A*. 2016;51:1034-1042.
38. Khan FM. Escherichia coli (E. *coli*) as an Indicator of Fecal Contamination in Water: A Review; 2020.
39. Islam M, Doyle MP, Phatak SC, Millner P, Jiang X. Persistence of enterohemorrhagic Escherichia coli O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *J Food Prot*. 2004;67:1365-1370.
40. Varela AR, Manaia CM. Human health implications of clinically relevant bacteria in wastewater habitats. *Environ Sci Pollut Res*. 2013;20:3550-3569.
41. Valdebenito M, Crumbliss AL, Winkelmann G, Hantke K. Environmental factors influence the production of enterobactin, salmochelin, aerobactin, and yersiniabactin in Escherichia coli strain nissle 1917. *Int J Med Microbiol*. 2006;296:513-520.
42. Petersen F, Hubbart JA. Physical factors impacting the survival and occurrence of Escherichia coli in secondary habitats. *Water*. 2020;12:1796.
43. Hernroth BE, Conden-Hansson A-C, Rehnstam-Holm A-S, Girones R, Allard AK. Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, Mytilus edulis: the first Scandinavian report. *Appl Environ Microbiol*. 2002;68:4523-4533.
44. Yilma M, Kiflie Z, Windsperger A, Gessese N. Assessment and interpretation of river water quality in Little Akaki River using multivariate statistical techniques. *Int J Environ Sci Technol*. 2019;16:3707-3720.
45. Ferens WA, Hovde CJ. Escherichia coli O157:H7: animal reservoir and sources of human infection. *Foodborne Pathog Dis*. 2011;8:465-487.
46. Jeong H, Kim H, Jang T. Irrigation water quality standards for indirect wastewater reuse in agriculture: a contribution toward sustainable wastewater reuse in South Korea. *Water*. 2016;8:169.
47. WHO U. Guidelines for the safe use of wastewater, excreta and greywater. *WHO Policy and Regulatory Aspects*; 2006:1.
48. Topalcengiz Z, Strawn LK, Danyluk MD. Microbial quality of agricultural water in Central Florida. *PLoS One*. 2017;12:e0174889.
49. USFDA. *FSMA Final Rule on Produce Safety: Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption*. USA FDA; 2022.
50. Luna-Guevara JJ, Arenas-Hernandez MMP, Martínez de la Peña C, Silva JL, Luna-Guevara ML. The role of pathogenic *E. coli* in fresh vegetables: Behavior, contamination factors, and preventive measures. *Int J Microbiol*. 2019;2019:2894328.
51. Matthews KR. Sources of enteric pathogen contamination of fruits and vegetables: future directions of research. 2013.
52. Faour-Klingbeil D, Murtada M, Kuri V, Todd ECD. Understanding the routes of contamination of ready-to-eat vegetables in the Middle East. *Food Control*. 2016;62:125-133.
53. Woldetsadik D, Drechsel P, Keraita B, Itanna F, Erko B, Gebrekidan H. Microbiological quality of lettuce (Lactuca sativa) irrigated with wastewater in Addis Ababa, Ethiopia and effect of green salads washing methods. *Int J Food Contam*. 2017;4:1-9.
54. Forslund A, Ensink JH, Markussen B, et al. Escherichia coli contamination and health aspects of soil and tomatoes (Solanum lycopersicum L.) subsurface drip irrigated with on-site treated domestic wastewater. *Water Res*. 2012;46:5917-5934.
55. Koch K, Bhushan B, Barthlott W. Diversity of structure, morphology and wetting of plant surfaces. *Soft Matter*. 2008;4:1943-1963.
56. Yi J, Huang K, Young GM, Nitin N. Quantitative analysis and influences of contact dynamics on bacterial cross-contamination from contaminated fresh produce. *J Food Eng*. 2020;270:109771.
57. Santore MM. Interplay of physico-chemical and mechanical bacteria-surface interactions with transport processes controls early biofilm growth: A review. *Adv Colloid Interface Sci*. 2022;304:102665.
58. Uyttendaele M, Jaykus LA, Amoah P, et al. Microbial hazards in irrigation water: standards, norms, and testing to manage use of water in fresh produce primary production. *Compr Rev Food Sci Food Saf*. 2015;14:336-356.
59. De Keuckelaere A, Jacxsens L, Amoah P, et al. Zero risk does not exist: lessons learned from microbial risk assessment related to use of water and safety of fresh produce. *Compr Rev Food Sci Food Saf*. 2015;14:387-410.
60. Gil MI, Selma MV, Suslow T, Jacxsens L, Uyttendaele M, Allende A. Pre- and postharvest preventive measures and intervention strategies to control microbial food safety hazards of fresh leafy vegetables. *Crit Rev Food Sci Nutr*. 2015;55:453-468.

61. Park S, Szonyi B, Gautam R, Nightingale K, Anciso J, Ivanek R. Risk factors for microbial contamination in fruits and vegetables at the preharvest level: a systematic review. *J Food Prot*. 2012;75:2055-2081.
62. Heaton JC, Jones K. Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. *J Appl Microbiol*. 2008;104:613-626.
63. Ensink JHJ, Mahmood T, Dalsgaard A. Wastewater-irrigated vegetables: market handling versus irrigation water quality. *Trop Med Int Health*. 2007;12:2-7.
64. Mcheik A, Awad A, Fadel A, Mounzer C, Nasreddine S. Effect of irrigation water quality on the microbial contamination of fresh vegetables in the Bekaa Valley, Lebanon. *Am J Agric For*. 2018;6:191-197.
65. Abdallah CK, Mourad KA. Assessing the quality of water used for vegetable irrigation in Tamale Metropolis, Ghana. *Sci Rep*. 2021;11:5314.
66. Machado-Moreira B, Richards K, Abram F, Brennan F, Gaffney M, Burgess CM. Survival of Escherichia coli and Listeria innocua on lettuce after irrigation with contaminated water in a temperate climate. *Foods*. 2021;10:2072.
67. Kirby RM, Bartram J, Carr R. Water in food production and processing: quantity and quality concerns. *Food Control*. 2003;14:283-299.
68. Barker SF, O'Toole J, Sinclair MI, Leder K, Malawaraarachchi M, Hamilton AJ. A probabilistic model of norovirus disease burden associated with greywater irrigation of home-produced lettuce in Melbourne, Australia. *Water Res*. 2013;47:1421-1432.