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Effect of Irrigation Water Quality on the Microbial Contamination of Fresh Vegetables in the Bekaa Valley, Lebanon

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Abstract: Irrigation water is probably the leading source of contamination of fresh vegetables in the world. In agricultural intensive areas, surface and groundwater resources are more likely to be exposed to be contaminated with zoonotic bacteria, given their close proximity to sources of faeces from livestock, dairy farms and wildlife. The aim of this study was to determine the role of irrigation water as a vehicle for the transmission of zoonotic bacteria of faecal origin and its contribution to the bacterial contamination of fresh vegetables to conclude with a need of risk management in rural areas. First, to determine the magnitude and the frequency of faecal contamination and the pathogens in the water source, the microbiological quality of the water resources used in irrigation in the studied area, including the upper Litani and the private wells located near its basin was investigated. In a second step, an assessment of the microbiological quality of the fresh vegetables irrigated from these water resources was done. A total of twelve different vegetables comprising spinach, parsley, cabbage and lettuce and 38 water samples were collected from both the well waters and the Litani River from the 5 studied sites and analyzed for a period of 5 months, to assess the microbial contamination level. Samples were analyzed for aerobic bacteria, total coliforms, faecal coliforms, *E.coli* and *S.aureus*. All vegetables sampled during the study period recorded high level of coliforms, *E.coli* and *S.aureus*. The microbial load recorded in the water samples was generally higher than that recorded in the vegetables. Since most of these vegetables are eaten fresh or slightly cooked, there is a concern on the public health that will be affected. Education of farmers and consumers on food safety has to be intensified to avert a possible outbreak.

Keywords: Faecal Coliforms, Irrigation Water, Well Water, Contaminated Vegetables

1. Introduction

Commercial and farmers generally irrigate their produce with water from nearby rivers, streams, ponds and wells which, in many cultivated areas worldwide, do not meet the required standard for irrigation [1]. These surface and groundwaters are easily contaminated by surface runoffs originating from non-point sources, animal and human wastes [2] and are not treated before they are used for irrigation [3].

The microbial quality of the irrigation water is critical because water contaminated with animal or human wastes can introduce pathogens into produce during pre-harvest and post-harvest [4]. Indirect or direct contamination of produce from water or water aerosols of persistent pathogens on harvested vegetables has been long recognized as a potential hazard [4-5]. The microbiological quality of the fresh vegetables is a significant concern for all stakeholders in the produce industry both local and international [6].

According to Henneberry, Piewthongngam and Qiang [7], the ten most common fresh vegetables consumed in the USA and other countries are broccoli, cauliflower, carrots, celery, lettuce, onions, tomatoes, cabbage, cucumbers and green peppers [7]. The microbiological quality of irrigation water is therefore paramount to the safety of fresh and minimally processed vegetables [8-9]. Ibenyassine *et al.* [10] reported that contaminated irrigation water and surface run-off water might be major sources of pathogenic microorganisms that contaminate fruits and vegetables in fields. Steele *et al.* [11] surveyed 500 irrigation water samples used for the production of fruits and vegetables in Canada and found that 25% of the water samples were contaminated with faecal *E.coli* and faecal *Streptococci*. River water used for both human and animal waste disposal poses a health risk due to contamination with *Salmonella* and *Listeria* when used for the irrigation of produce [12-13]. Combarro *et al.* [12] isolated different *Listeria* species from river water in Spain. The specie most isolated was *L.monocytogenes*, followed by *L.seeligeri*, *L.velshimeri* and *L. ivanovii*. A study which was done by Halablab *et al.* [14] and Temsah *et al.* [15] on irrigated vegetables from the Litani River in the Bekaa region near the Karaoun Lake in Lebanon showed a high contamination of the irrigated plants from the Litani River.

Bekaa valley, the largest agricultural area in Lebanon, is facing today a deterioration in its water resources, including surface and groundwaters [16], which became a growing concern for public health [17]. The deterioration in the water resources in the Bekaa valley includes primarily the Litani River, the largest River in Lebanon and water sources enriching it which are of great importance agriculturally and touristically. This deterioration in the quality of water, including irrigation water, became associated with the consumption of contaminated vegetables and with the increase in the outbreaks of foodborne infections [18-19].

This increase in foodborne infections is the result of many parameters including (1) changes in dietary habits including the increase in the consumption of fresh or minimally processed fruits and vegetables and the increase in using salad bars and meals eaten outside the home [20-21], (2) changes in the production and the processing methods; (3) the agronomic and harvesting procedures; (4) the distribution and the consumption patterns and practices; (5) the increase in susceptible population with changes in human demographics and behavior; (6) a change to a life style of convenience and consumer demands regarding healthy food with no chemical preservatives and with an extended shelf life [22].

It must be emphasized also that vegetables can become contaminated with foodborne pathogens in various ways during production, harvesting, processing, transportation, in retail and food services and even at home [23].

Thus, the aim of this study was to determine the bacterial quality of irrigation water of both the Litani River and the adjacent wells and their subsequent contribution to the bacterial contamination of fresh vegetables.

2. Materials and Methods

2.1. Sample Collection

The study was conducted on five sites (5 villages) in the Bekaa region through which passes the Upper Litani River. The five studied sites are Housh Barada, Ain Sawda, Bednayel (Figure 1), Deir Zanoun (Figure 2) and Housh Rafka (Table 1). The studied sites are characterized by the presence of a large number of chicken farms, cow farms and dairy farms in addition to a high agricultural activity. These villages were selected to represent the water pollution potential on the contamination of the irrigated vegetables. A total of 38 water samples were collected from the 5 studied villages. Water samples include water collected from the Litani River passing through these villages and water collected from wells present in these villages and used in irrigation. Both water sources were used by the farmers for irrigation. In addition to the water samples, a total of twelve (12) different vegetables were also selected from the five studied villages including spinach, parsley, cabbage, lettuce, mint, radish, coriander and onions. The selection of these 5 villages was based on the presence of cultivated lands irrigated either from the Litani River and/or from the wells. The 5 studied villages are presented in Figure 3.

Figure 1. *Site at Bednayel.*

Figure 2. *Site at Deir Zanoun.*

Water samples were aseptically collected from the 5 studied sites and studied over a period of 5 months from February to July 2018. The temperature, pH and electrical conductivity of the irrigation water were analyzed in situ using a pH/ORP, CD/TDS Meter YK-2005WA. Irrigation water was collected in 200 mL sterilized glass bottles preserved with ice during transport to the laboratory and immediately analyzed microbiologically after return to the laboratory. At the same time, vegetables were collected in sterile plastic bags for the microbiological analysis with the water samples in the laboratory.

2.2. Laboratory Analysis

Irrigation water and vegetable samples were examined for the presence of aerobic bacteria, total and faecal coliforms, *E.coli* and *S. aureus*. For the microbiological analysis of the vegetables, 50 g of each sample were weighed and blended in 100 mL sterile physiological water and under sterile conditions. The blender was carefully disinfected between the preparations of the successive samples to prevent any cross contamination. The homogenates were collected in sterile bottles. After their collection, aliquots (1 mL) of each prepared homogenate were serially diluted using sterile physiological water. At this stage, samples were ready to be inoculated in the prepared media and to be incubated for the detection of the presence of any contamination. Plate count agar was used for the detection of total aerobic bacteria after their incubation at 37°C for 24-48hrs. After their incubation, typical colonies on the media will be enumerated and colony counts per 1 g sample will be determined. Colony counts will be converted into $\log_{10}CFU.g^{-1}$. Macconkey agar medium was used to detect the presence of total and faecal coliforms with plates inoculated and incubated for 24-48 hrs at 37°C and 44°C, respectively [24]. Trypton bile X-glucuronide (TBX) agar was used to detect the presence of *E.coli* and the inoculated plates were incubated from 24-48 hrs at 37°C. For the isolation and enumeration of *S.aureus*, Baird Parker Agar containing egg yolk emulsion was used and the inoculated plates were incubated at 37°C for 24 hrs followed by a coagulase test to test the presence of coagulase -positive staphylococci [25].

2.3. Statistical Analysis

Statistical analysis, including geometric means, standard deviations, minimum and maximum values were calculated using SPSS. Total aerobic bacteria, total coliforms, faecal coliforms, *E.coli* and *S.aureus* loads on vegetables and water were normalized by log transforming the raw data before it was analyzed.

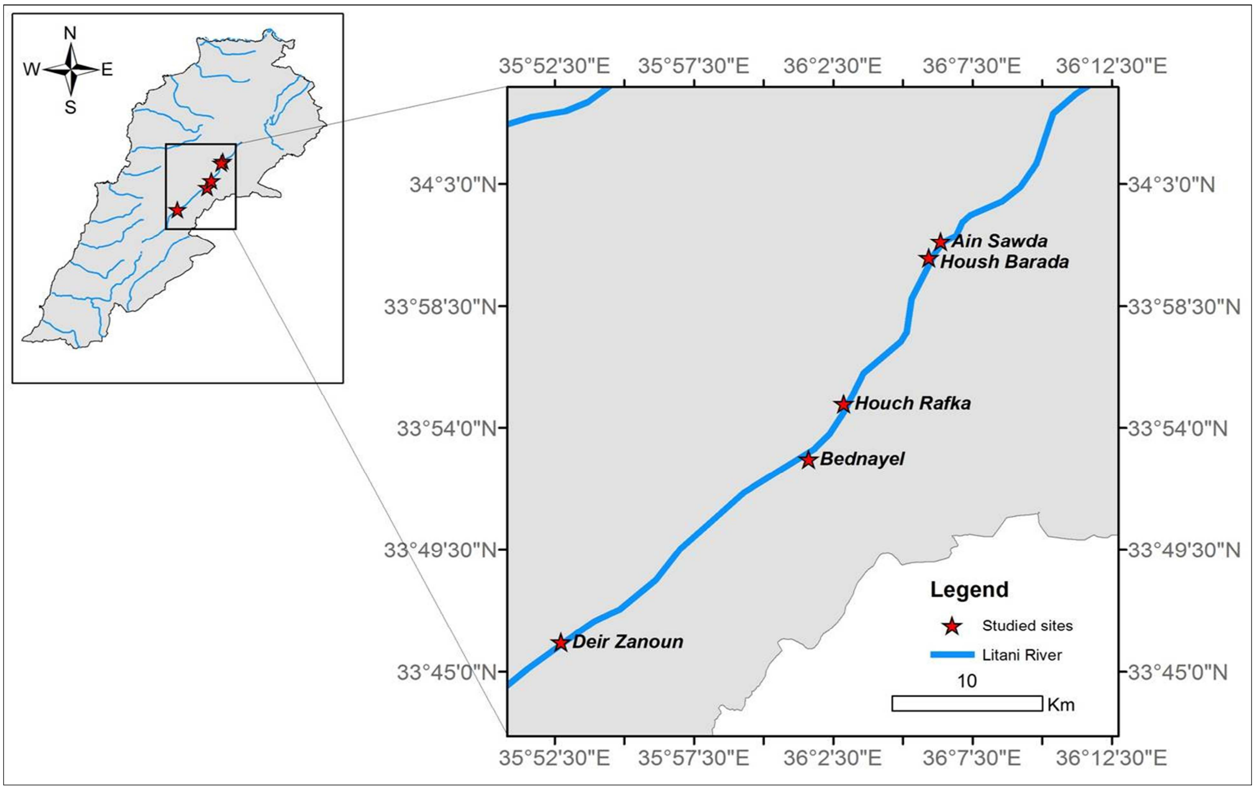

Figure 3. *Map representing the sampling sites from the 5 studied villages in the Bekaa valley.*

3. Results and Discussion

The consumption of fresh fruits and vegetables is increasing as consumers strive to eat healthy diets and benefit from the year-round availability of these products that up until recently were considered to be seasonal. Global trade in fruits and vegetables and changing horticultural practices have enabled this year-round abundance to be possible, as well as adding new varieties of fresh produce to the market.

During the last few decades pre-prepared minimally processed fruits and vegetables have become popular among the European consumers. These products include pre-washed pre-cut salads items, chopped crudités, sprouted seeds, grated vegetables, prepared fruits salads, or fruit combinations. Most of these products are generally eaten without further processing. Some products are packed in modified atmospheres to provide extension of shelf life both in relation to the potential acceptable quality and safety of the product.

Microbiological quality of the irrigation water samples

During our study, a total of 33 vegetable samples and 38 water samples were collected from 5 villages of the Bekaa valley. The 38 water samples include 33 well water samples taken from the same places where the vegetable samples were taken and 5 water samples taken from the Litani River passing through the 5 villages. All the vegetable samples taken were irrigated from well waters located near the Litani River (Table 1) and passing through the five villages.

The microbiological analysis of the well water samples indicated that all the samples taken from the 5 sites were contaminated with total coliforms and most of them were contaminated with faecal coliforms, *E.coli* and *S.aureus* (Table 2). At Deir Zanoun, about 92.4% of the well water samples were contaminated with faecal coliforms and 78.7% were contaminated with *S.aureus*.

Table 1. *Vegetable samples collected from the 5 studied sites and irrigated from well waters in these locations and near to the Litani River.*

Sample type	No of samples	Sample location	Source of irrigation
Lettuce, coriander, mint, radish, dandelion	6	Housh Barada	Well water
Wheat, potato, onions	6	Ain Sawda	Well water
Mint, wheat, cabbage, peas, radish, coriander, dandelion	9	Deir Zanoun	Well water
Mint, potato, wheat	6	Bednayel	Well water
Wheat, spinach, lettuce, mint	6	Housh Rafka	Well water

At Bednayel, 85.5% of the well water samples were contaminated with faecal coliforms and 65.4% of these samples were contaminated with *S.aureus*. At Housh Rafka, 45.2% of the well water samples taken from this site were contaminated with faecal coliforms and 35.2% of these samples showed their contamination with *S.aureus*. At Ain Sawda, 38% of the well water samples showed their contamination with faecal coliforms and 30% of these same samples showed their contamination with *S.aureus* and at Housh Barada, 17.5% of the well water samples showed their contamination with faecal coliforms and 14.5% of these same samples were contaminated with *S.aureus*.

Table 2. *Percentage of total aerobic, total coliforms, faecal coliforms, E.coli and S.aureus isolated from different well water samples and collected from different locations.*

Samples contaminated					
Sample location	**Total aerobic bacteria (%)**	**Total coliforms (%)**	**Faecal coliforms (%)**	***E.coli (%)***	***S.aureus (%)***
Deir Zanoun	100	100	92.4	82.7	78.7
Bednayel	100	100	85.5	80.5	65.4
Housh Rafka	100	100	45.2	35.00	35.2
Ain Sawda	100	100	38.00	30.7	30.00
Housh Barada	100	100	17.5	15.3	14.5

This high contamination in the well water samples may be due to the infiltration of domestic wastewater discharges and/or livestock manure. In addition to it usage for irrigation, this type of water is used by the citizens for domestic purposes but not for drinking and cooking. What should be noted is that the well water samples were taken from the same sites where the vegetables were irrigated from these waters and taken for analysis and therefore the microbiological contamination of these waters could be a source of bacterial contamination to the cultivated vegetables.

What should be noted also is that the microbiological analysis of the water samples taken from the Litani River passing through the five studied sites showed a high bacterial contamination (total coliforms, faecal coliforms, *E.coli* and *S.aureus* (Table 3). The results of the microbiological contamination of the Litani River confirmed what was presented by Haydar *et al.* [26]. This river is very near to the cultivated region, it passes through the cultivated lands and it is known as it is highly polluted that is why the farmers said that they do not use the river water for irrigation but during sampling the citizens living near to the cultivated lands said that this highly contaminated water is used by the farmers for the irrigation.

Microbiological quality of the vegetable samples

Vegetables may be contaminated with pathogenic microorganisms during growth in the field, during harvesting, post-harvesting, handling, processing and distribution. Therefore, vegetables may act as a reservoir for many microorganisms from which they will be colonized inside these vegetables and infect susceptible host. Fruits and vegetables that have been contaminated with pathogens either from the environment or from human or animal faeces or through storage, processing and handling could potentially cause disease [27]. Table 4 presents the minimum, maximum and the mean values for the results of the microbiological analysis done on the 33 vegetable samples taken from the five studied sites. These results were calculated using the geometric mean ($\log_{10}$CFU.g^{-1}) bacterial indicator concentrations of each of the different vegetable samples. The results obtained and presented in this table showed that all of the vegetables which were irrigated with contaminated well waters were also contaminated with coliforms, *E.coli* and *S.aureus* indicating that irrigation water is a source of contamination to the irrigated vegetables where the transported pathogens from contaminated irrigating water may survive in soil and in crops which in turn, will be transported to the consumers and may cause numerous diseases. These results showed that lettuce, mint, dandelion, spinach, coriander and radish have a higher microbial loads of coliforms and *S.aureus*. The highest level of contamination of total coliforms, faecal coliforms, *E.coli* and *S.aureus* were recorded on lettuce. The mean levels of total coliforms, faecal coliforms, *E.coli* and *S.aureus* were 6.52; 6.0; 5.0 and 5.3 $log_{10}CFU.g^{-1}$ fresh weight, respectively. *E.coli* detected in lettuce ranged from 4.3 to 5.7 $log_{10}CFU.g^{-1}$ fresh weight with a mean of 5.0$log_{10}CFU.g^{-1}$.

The primary source of contamination of these vegetables is from irrigated water. But also many sources of contamination may be responsible including "daefacation' from humans and animals on marginal lands around the farms and from the application of fresh poultry manure on farm plots [28-29].

Bacterial counts on lettuce, spinach and dandelion were higher than that on the other vegetables. This high bacterial counts is due to the larger surface area exposed to irrigation water. The contamination of these foods by these pathogenic microorganisms may present a potential health hazard to the consumers and the consumption of contaminated vegetableshas been associated with gastrointestinal diseases like typhoid, cholera and dysentery [14].

Table 3. *Physical and Microbiological characteristics of the Litani River water at the 5 studied site.*

Characteristics							
Studied sites	**Temp (°C)**	**pH**	**EC (μs/cm)**	**Total coliforms**	**Faecal coliforms**	***E.coli***	***S.aureus***
Deir Zanoun	22.2	7.87	1894	8.74	6.80	6.15	3.42
Bednayel	20.4	7.5	1622	7.92	6.87	6.3	4.70
Housh Rafka	20.3	7.74	1343	6.47	5.72	4.78	3.28
Ain Sawda	19.63	7.43	584	6.85	5.74	4.85	4.32
Housh Barada	22.9	7.74	1343	6.54	5.52	3.47	4.75

The units for total aerobic bacteria, total coliforms (TC), Faecal coliforms (FC), *E.coli* and *S.aureus* are in log_{10} $CFU.g^{-1}$ fresh weight

According to the Hazard Analysis and Critical Control Points-Total Quality Management (HACCP-TQM) Technical microbial quality for raw foods guidelines [14-30], food containing less than 4; 4-6.69; 6.69-7.69 and greater than 7.69 $log_{10}CFU.g^{-1}$ (aerobic plate count) are rated as good, average, poor and spoiled food, respectively. Based on these characteristics, the results obtained from the present study showed that lettuce samples could be regarded as spoiled vegetable food and both dandelion and spinach could be considered as poor vegetables food and the consumption of lettuce, dandelion and spinach as raw salad vegetables can serve as a vehicle of these foodborne pathogens. All lettuce samples, which were collected from the different studied sites showed that they are the most contaminated samples with total aerobic bacterial count ranging between 7.84 and 8.75 $log_{10}CFU.g^{-1}$. In addition, this contamination of the vegetables by these pathogens might be from irrigation water or from faecal contamination from humans or animals [14]. In addition, contamination of lettuce samples by *S.aureus* was also high and ranging from 4.75 to 5.95 $log_{10}CFU.g^{-1}$.

This high microbial load on lettuce samples may be due to their large surface area with many folds and fissures which provide a good shelter for microorganisms and the fragility of leaves allow the penetration and the reproduction of bacteria in their inner tissues.

At the end of this study, it should be noted that during sampling of both the vegetables and the water, people living in the studied region told us that sometimes the farmers pump the water of the Litani River to irrigate the cultivated lands and the farmers refused saying that they use this water. After asking them, people living in this region told us that they suffered many times from many health problems such as gastrointestinal diseases and they know that these problems may be from the pollution of the Litani River. This river passes through the cultivated lands in the different studied sites and it is known for it high pollutionnal load. This river is a source of contamination to the soil, the nearest well waters and the cultivated lands.

Table 4. *Mean levels of total aerobic bacteria, total coliforms, faecal coliforms, E.coli and S.aureus in different types of vegetable samples irrigated from the well waters.*

Samples contaminated						
Sample type	**Sampling location**	**Total aerobic bacteria (%)**	**Total coliforms (%)**	**Faecal coliforms (%)**	***E.coli*** **(%)**	***S.aureus*** **(%)**
Lettuce	Housh Barada, Housh Rafka	(7.84-8.75) Mean=8.29	(6.25-6.80) Mean=6.52	(5.51-6.50) Mean=6.005	(4.30-5.70) Mean= 5	(4.75-5.95) Mean=5.3
Coriander	Housh Barada	(3.87-4.47) Mean=4.17	(2.58-3.83) Mean=3.20	(2.20-2.50) Mean=2.35	(1.74-2.32) Mean=2.03	(2.27-2.58) Mean=2.4
Mint	Housh Barada, Deir Zanoun, Bednayel, Housh Rafka	(2.72-3.50) Mean=3.11	(2.12-2.36) Mean=2.24	(1.30-2.10) Mean=1.7	(1.17-1.75) Mean=1.46	(2.41-2.8) Mean=2.6
Radish	Housh Barada, Deir Zanoun	(3.54-3.82) Mean=3.68	(2.36-3.47) Mean=2.91	(1.50-2.34) Mean=1.92	(1.22-2.16) Mean=1.69	(2.24-2.35) Mean= 2.2
Dandelion	Housh Barada, Deir Zanoun	(6.35-7.74) Mean=7.04	(6.28-6.94) Mean=6.61	(5.27-6.18) Mean=5.72	(3.34-4.62) Mean=3.98	(4.40-4.74) Mean= 4.5
Wheat	Ain Sawda, Deir Zanoun, Housh Rafka	(2.35-2.73) Mean=2.54	(1.56-1.83) Mean=1.69	(1.24-1.43) Mean=1.33	(1.18-1.30) Mean=1.24	(1.21-1.73) Mean= 1.4

Samples contaminated							
Sample type	**Sampling location**	**Total aerobic bacteria (%)**	**Total coliforms (%)**	**Faecal coliforms (%)**	***E.coli* (%)**	***S.aureus* (%)**
Potato	Ain Sawda, Bednayel	(2.28-2.56) Mean=2.42	(2.15-1.73) Mean=1.94	(1.26-1.68) Mean=1.47	(0.70-1.10) Mean=0.90	(1.15-1.23) Mean=1.1
Onions	Ain Sawda	(1.72-2.25) Mean=1.98	(1.24-1.65) Mean=1.44	(0.89-1.28) Mean=1.08	(0.38-0.76) Mean=0.57	(1.47-1.56) Mean=1.5
Cabbage	Deir Zanoun	(3.25-4.73) Mean=3.99	(2.54-3.25) Mean=2.89	(2.10-2.25) Mean=2.17	(0.52-1.80) Mean=1.16	(2.45-2.63) Mean=2.5
Peas	Deir Zanoun	(3.37-4.78) Mean=4.07	(2.15-3.34) Mean=2.74	(1.15-1.34) Mean=1.24	(0.73-0.97) Mean= 0.85	(0.63-0.87) Mean=0.7
Spinach	Housh Rafka	(6.20-7.76) Mean= 6.98	(5.52-6.30) Mean=5.91	(4.35-5.53) Mean=4.94	(3.18-4.63) Mean=3.90	(2.43-2.70) Mean=2.5

The units for total aerobic bacteria, total coliforms (TC), Faecal coliforms (FC), *E.coli* and *S.aureus* are in $\log_{10}$ CFU.g^{-1} fresh weight

4. Conclusion and Recommendations

The deterioration of the quality of the surface and groundwater resources is one of the major threats the country is facing today. The bacteriological contamination of these water resources, originating from the absence of poorly maintained sanitation facilities, became widespread and deteriorating large cultivated lands. People eating fruits and vegetables from the contaminated lands, stand a high chance of developing gastrointestinal diseases. To prevent such these outbreaks, efforts have to be made to stop the contamination of surface and groundwater resources, efforts have to be made also to discourage farmers from the use of the Litani water for irrigation. This River which is not only chemically contaminated and is the direct responsible for the many cases of cancer affecting the region, it is also biologically contaminated. If any surface water is used for irrigation, it should be tested for *E.coli* on a regular schedule to monitor the microbiological quality and any changes that may occur due to unusual contamination events. If well waters are used, farmers should be sure that the wells are capped and properly constructed and should be tested at least once a year to monitor their microbiological quality. If they continue usage of contaminated water, then they have to be educated on good agricultural practices such as reducing drip or surface irrigation which should be used only when possible to prevent direct wetting of the plants or ripe fruits or vegetables. Legislation against the use of contaminated water for irrigation could be improved as well as education of farmers on pre-harvesting practices that will help reduce microbial loads on the vegetables.

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