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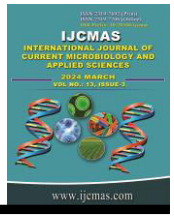

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Effect of Sewage-Contaminated Irrigation Water on the Bacterial Quality of Ready-To-Eat Fresh Vegetables; Watercress (*Eruca sativa*) and Lettuce (*Lactuca sativa*)

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A B S T R A C T

In Egypt, some people illegally dump untreated sewage into irrigation canals. Farmers use these polluted canals and the water from the Bahr El-Baqar drain to irrigate farmlands. The bacterial content of irrigation water, agricultural soil, and ready-to-eat vegetables gathered from farmlands was evaluated. Watercress (*Eruca sativa*) and lettuce (*Lactuca sativa*) were selected since they are major ready-to-eat vegetables in most Egyptian households and restaurants. A total of 80 samples (20 irrigation water, 20 farmland soil, 20 watercress, and 20 lettuce) were collected from four farmlands irrigated with partially treated sewage from the Bahr El-Baqar drain. Another 80 similar samples were collected from farmlands irrigated with canals polluted with illegally dumped untreated sewage. The efficacy of four different watercress and lettuce washing methods was also assessed. *E. coli*, *E. coli* O157:H7, and *Salmonella* sp. showed significant survival on watercress and lettuce for over 15 days. Regardless of the tested washing strategies, a vinegar-based washing strategy can reduce bacterial levels. In conclusion, using irrigation water polluted with untreated or partially treated sewage contaminated the ready-to-eat fresh vegetables with pathogenic bacteria, which is considered a high risk to human health.

Introduction

In developing countries, some urban and semi-urban villages and cities use partially treated or diluted wastewater for irrigation purposes (Raja *et al.,* 2015). In Egypt, the River Nile and its canals are the main sources of freshwater required for drinking and irrigation purposes. However, for several decades, large volumes of untreated wastewater have been discharged into these canals, leading to the deterioration of the water quality

(Abdel-Satar *et al.,* 2017). According to Abd Abdallah (2014), the main source of pollution in the River Nile is the untreated wastewater from open drains carrying agricultural return flows and domestic and industrial wastewater discharge into the canals. Consequently, farmers use the polluted water from these canals for irrigation purposes (Abdel-Satar, 2005; Abdallah, 2014). Watercress (*Eruca sativa*) and lettuce (*Lactuca sativa*) are popular and indispensable ingredients in the daily menus of Egyptians' home and restaurant meals, mainly as green salad ingredients. These ready-to-eat vegetables have high nutritional value and are great sources of minerals, vitamins, and fibers (Said, 2012). However, most urban farmers who irrigate their vegetables with water from the polluted River Nile canals, which contain untreated and/or partially treated sewage, supply watercress and lettuce to consumers. Consequently, these raw vegetables are more susceptible to microbial and chemical contamination (Blaak *et al.,* 2015).

Watercress and lettuce grown in farms irrigated with fecal-containing surface water require special attention since potential pathogenic microorganisms such as bacteria, protozoa, helminths, and viruses that come into contact with vegetables may persist for some time on the surface of the plants and could cause infections for consumers (Beuchat, 2002; Aruscavage *et al.,* 2006; Qadir *et al.,* 2010). Previous studies reported that pathogenic microorganisms could survive for different periods (days, weeks, and months) on the surface of vegetables irrigated with water containing untreated or partially treated wastewater (Mrayyan, 2005; Keraita *et al.,* 2007; Gemmell and Schmidt, 2012). In addition, heavy metals released into surface water from industrial wastewater can be a potential risk factor (Weldegebriel *et al.,* 2012). Arora *et al.,* (2008) indicated that heavy metals could easily accumulate in the edible parts of leafy vegetables irrigated with wastewater compared to fruit or grain crops. Moreover, toxic heavy metals such as lead (Pb), cadmium (Cd), Nickel (Ni), and Chromium (Cr) can accumulate in soils irrigated with wastewater effluent (Letshwenyo and Mokokwe, 2020). Studies have reported different bacterial indicator loads in vegetables irrigated with treated wastewater. For instance, high levels of total coliforms were observed in spinach, followed by radish and eggplants. The accumulation of high counts of bacterial indicators, such as total coliforms and *E. coli*, was reported in the soil and roots of cauliflower and eggplant irrigated with wastewater (Letshwenyo and Mokokwe, 2020). Irrigating agricultural lands with untreated or partially treated wastewater can contaminate soil and groundwater with fecal pathogens such as *Salmonella* sp., *Shigella* sp., protozoa and enteric viruses (Lonigro *et al.,* 2016). Khalil *et al.,* (2015) reported a link between the increasing number of foodborne outbreaks of human illness and the consumption of raw vegetables contaminated with gastrointestinal pathogens. Fecal coliforms, *E. coli*, and *Enterococci* are used as bacterial indicators of fecal pollution since it is challenging to identify all potentially pathogenic bacteria in water sources and on grown vegetables. When these bacterial indicators are present, it is typically considered that additional pathogenic bacteria such as pathogenic *E. coli*, *Salmonella* sp., *Staphylococcus aureus* and *Shigella* sp. are also present. These pathogenic bacteria cause gastroenteric diseases by consuming ready-to-eat vegetables irrigated with water contaminated with fecal matter (WHO, 2006; Maimon *et al.,* 2010; Khattab *et al.,* 2016). This study used fecal bacterial indicators and some selected pathogenic bacterial species (*Salmonella* sp. and *E. coli* O157:H7) to assess the bacterial quality of irrigation canals branched from the River Nile, which receives wastewater from nearby drains or via illegal discharging of untreated wastewater by villagers. From a bacteriological perspective, the study investigates the effect of irrigating with such contaminated water on the soil and the surface of ready-to-eat vegetables (watercress and lettuce). Additionally, the study investigates the survival of *E. coli*, *E. coli* O157:H7, and *Salmonella* sp. on watercress and lettuce samples using a bacterial challenge test. Furthermore, the study examines the effect of different washing strategies for removing fecal bacterial indicators and pathogenic bacteria from watercress and lettuce leaves.

Materials and Methods

Samples

During the current study, two samples were collected from farmlands in Faqous City, Sharkia governorate, Egypt. Set I comprised a total of 80 samples, including 20 samples each of irrigation water, farmland soil, watercress, and lettuce, collected from four different farmlands with five runs each. The first set of farmlands is irrigated by a canal that is contaminated with wastewater from the Bahr El-Baqar drain (Fig. 1). This drain receives wastewater from three main sources: (a) treated wastewater from the El-Gabal El-Asfar and El-Berka wastewater treatment plants (located northeast of Greater Cairo), (b) drainage of cultivated lands (> 1.2

million feddans), and (c) raw and treated industrial wastewater from Abu-Zaabal, Shoubra El-Khiema, El-Khanka, and other parts of the industrial zone (northeastern suburbs of Greater Cairo). Set II comprised 80 samples, including 20 samples each of irrigation water, soil, watercress, and lettuce, collected from another four farmlands with five runs each. The second set of farmlands is irrigated by a canal that is contaminated with untreated sewage that is illegally discharged by villagers (Fig.2).

Sampling

Irrigation water samples were collected in 1-L sterile polyethylene terephthalate (PET) bottles from the source point of the farmlands. Soil samples weighing 250 g were collected using ethanol-sterilized scoops at a depth of 15–20 cm. The soil samples were then sieved through a sterilized 2-mm sieve to remove plant and gravel debris and placed in sterile polyethylene bags. Watercress and lettuce samples were harvested and transferred to sterile polyethylene bags. All samples were stored in an icebox at 4°C and transported to the laboratory for further experiments.

Preparation of samples for Bacterial analysis

Irrigation water samples

Ten milliliters of each irrigation water sample was aseptically pipetted into a 200 ml sterile Erlenmeyer flask, and 90 ml of quarter-strength Ringer's solution was added to dilute each sample tenfold. A subsequent decimal dilution (up to 10^{-6}) was performed using the same diluent (quarter-strength Ringer's solution).

Soil samples

Using an orbital shaker, 10 g of each soil sample was aseptically transferred into a 200 ml Erlenmeyer flask containing 90 ml of quarter-strength Ringer's solution, and a subsequent decimal dilution (up to 10^{-6}) was performed using the same diluent (quarter-strength Ringer's solution) at room temperature.

Watercress and lettuce samples

Separately, 10 g of both outer and inner leaves of watercress and lettuce samples were added to a 200 ml Erlenmeyer flask containing 90 ml of quarter-strength Ringer's solution, and the mixture was shaken at 200 rpm for 10 min at room temperature using an orbital shaker. A subsequent decimal dilution (up to 10^{-6}) was performed using the same diluent (quarter-strength Ringer's solution).

Bacterial Examination of Samples

The serial dilution technique and subsequent membrane filtration method were performed according to APHA (2017), and the bacterial counts were expressed as colony-forming units (cfu) per milliliter for irrigation water samples and per gram for soil, watercress, and lettuce samples.

Fecal Bacterial Indicators

Fecal coliforms, *E. coli*, and *Enterococci* were detected in all samples, including irrigation water, soil, watercress, and lettuce samples. For the detection and enumeration of fecal coliforms, *E. coli*, and *Enterococci*, m-FC agar (Difco, USA), modified mTEC agar (Difco, USA), and m-enterococcus agar (Himedia™, India) media were used, respectively. Fecal coliforms appeared as light to dark blue colonies on m-FC agar after incubation at 44–45°C in a water bath for 24 h. *E. coli* appeared as red or magenta on modified mTEC agar after incubation at 44–45°C in a water bath for 24 h. *Enterococci* appeared as light to dark red colonies on m-enterococcus agar after incubation at 35°C for 48 h.

Pathogenic bacteria

E. coli O157:H7 and *Salmonella* sp. were detected in all the samples mentioned earlier. MUG EC O157 Agar (Himedia™, India) and HiCrome™ *Salmonella* Agar (Himedia, India) were used for the rapid and selective detection of *E. coli* O157:H7 and *Salmonella* sp., respectively. *E. coli* O157:H7 appeared as colorless colonies with negative fluorescence under ultraviolet (UV) light (366 nm) after incubation at 37°C for 24 h. *Salmonella* sp. appeared as light purple colonies with halos after incubation at 37°C for 24 h.

Survival of bacterial indicators and pathogens

Processing of watercress and lettuce samples

Single colonies of *E. coli*, *E. coli* O157:H7, and *Salmonella* sp. were picked up from Petri dishes of the

previous step, which involved bacterial examination of samples before the microbial challenge test. The watercress and lettuce leaves were prepared according to the instructions provided by Feroz *et al.,* (2013) and Noor *et al.,* (2015), with slight modifications. To prepare 50 g each of watercress and lettuce leaves, they were cleaned with distilled water and 95% ethanol. Then, 10 g of each sample was chopped, and 90 ml of buffered peptone water (BPW) was added. The mixture was centrifuged at 5000 rpm for 5 min, and the supernatant was removed and rinsed with BPW. The second round of centrifugation was performed, and the process was repeated five times.

The resulting pellets were washed twice with 95% ethanol and then washed with 70% ethanol. The samples were washed three times with sterilized distilled water to remove the remains of ethanol and BPW from the samples. Finally, 100 µl of each sample was placed on the surface of sterilized nutrient agar dishes to confirm the complete elimination of contaminating bacteria. The absence of colonies on the nutrient agar media after incubation at 37°C for 24 h confirmed that the samples were ready for the bacterial challenge test.

Bacterial challenge test

A loopful (~10^7 cells) of each pure culture of *E. coli*, *E. coli* O157:H7, and *Salmonella* sp. were transferred separately into tubes containing 9 ml of sterile saline solution. Then, 100 µl of the bacterial suspension was added to 10 ml of the leaf suspensions (watercress and lettuce), resulting in an initial load of ~10^5 cfu/g. The challenge test media ratio (inoculum versus leaf suspensions) was balanced to be enough to sustain the inoculated bacterial growth and survival, as detailed by Jay (2000), with a ratio of 1:1000 (v/v). Uninoculated samples were used as controls. The inoculated samples were enumerated every 24 h up to 15 days using the standard plate count technique (APHA, 2017). The experiments were conducted in triplicate, and the reduction percent was calculated using the following formula:

$$\text{Reduction (\%)} = \frac{\text{(Load I - Load F)}}{\text{Load I}} \times 100$$

where load *I* is the initial load and load *F* is the final load.

Effect of washing strategies

The study examined the effect of different washing methods on removing bacterial indicators and pathogens from raw watercress and lettuce. One-hundred grams of watercress and lettuce leaves (outer and inner) were cut into pieces using a sterile knife to determine the initial counts of fecal bacterial indicators and pathogenic bacteria. Another 100 g of each watercress and lettuce leaf was immersed in containers and subjected to four different washing methods: W0 (control), W1 (tap water washing and rinsing for 2 min), W2 (tap water washing and rinsing for 2 min, then submerged in salt solution (30 ppm) for 1 min), W3 (tap water washing and rinsing for 2 min, then submerged in a vinegar solution (12000 ppm) for 1 min), and W4 (tap water washing and rinsing for 2 min, then submerged in a combined solution of vinegar (12000 ppm) and salt (30 ppm) for 1 min). The samples were analyzed for *E. coli*, *Salmonella* sp., and *E. coli* O157:H7 immediately after washing and compared to the original counts (i.e., without washing). After washing, the watercress and lettuce leaves were transferred to a sterile dry container using a sterile spatula and air-dried for 5 min. Then, 20 g of each watercress and lettuce sample was washed by shaking thoroughly with 230 ml of quarter-strength Ringer's solution to detect bacterial counts. The experiment was conducted in triplicate (n = 3).

Results and Discussion

Characterization of irrigation water sources

Table 1 summarizes the physicochemical characterization of the two irrigation water sources, namely the Bahr El-Baqar drain and the irrigation canal contaminated with wastewater. The physicochemical parameters measured include pH, temperature, total dissolved solids (TDS), total suspended solids (TSS), biological oxygen demand (BOD_5), chemical oxygen demand (COD), total nitrogen (TN), and oil and grease (O&G). All physicochemical parameters were measured following the guidelines established by APHA (2017). As shown in Table 1, the results indicate that both the Bahr El-Baqar drain and the irrigation canal suffer from water pollution. Notably, Bahr El-Baqar drain samples exhibited higher pollution levels across all measured parameters than the irrigation canal samples. The main source of pollution in the irrigation canal is the illegal discharge of raw wastewater.

Concentrations of fecal bacterial indicators and pathogenic bacteria

Bahr El-Baqar drain

Figure 3 summarizes the log average counts of fecal bacterial indicators (fecal coliforms, *E. coli*, and *Enterococci*) in irrigation water (Bahr El-Baqar drain), soil, watercress, and lettuce samples collected from the four farming sites. The irrigation water from Bahr El-Baqar drain showed the highest counts of fecal bacterial indicators, with $\log_{10}$ counts ranging from 6.64–6.78, 5.46–6.60, and 4.41–5.15 cfu/100 ml for fecal coliforms, *E. coli*, and *Enterococci*, respectively, across all four farmlands. The counts of fecal indicators decreased in soil samples, with $\log_{10}$ counts of 5.83–6.12, 5.08–5.73, and 3.93–4.65 cfu/100 ml for fecal coliforms, *E. coli*, and *Enterococci*, respectively. The $\log_{10}$ counts of fecal bacterial indicators in watercress and lettuce samples showed only a slight difference, with a slight increase observed in lettuce samples, which may be attributed to the coiled shape of lettuce leaves that can harbor a higher number of fecal bacterial indicators.

Figure 4 summarizes the $\log_{10}$ counts of pathogenic bacteria (*Salmonella* sp. and *E. coli* O157:H7) in samples collected from irrigation water (Bahr El-Baqar drain), soil, watercress, and lettuce. The pattern of pathogenic bacterial counts was consistent with that of the bacterial indicators, with the highest counts observed in irrigation water samples, followed by soil samples, lettuce, and the lowest counts in watercress samples. Additionally, the $\log_{10}$counts of *Salmonella* sp. were found to be higher than those of *E. coli* O157:H7 across all samples. The $\log_{10}$ counts of *Salmonella* sp. ranged from 1.5–6.0 cfu/100 ml, while the $\log_{10}$ counts of *E. coli* O157:H7 ranged from 1.2–2.9 cfu/100 ml.

Irrigation canal water

Figure 5 depicts the $\log_{10}$ counts of fecal bacterial indicators (fecal coliforms, *E. coli*, and *Enterococci*) in irrigation water (canal water), soil, watercress, and lettuce samples collected from the four farming sites. The canal water samples showed the highest concentrations of fecal bacterial indicators, followed by soil samples, lettuce samples, and the lowest counts observed in watercress samples. Specifically, the $\log_{10}$ counts of fecal coliforms ranged from 5.41–5.66, 4.66–5.07, 2.88–3.9, and 2.57–3.71 cfu/100 ml for canal water, soil, lettuce, and watercress samples, respectively. Similarly, *E. coli* $\log_{10}$ counts ranged from 4.55–4.66, 3.73–4.32, 2.35–2.95 and 2.13–2.65 cfu/100 ml, respectively. Moreover, *Enterococci* showed a similar pattern, with $\log_{10}$ counts ranging from 3.86–4.14 (canal water), 3.41–3.78 (soil samples), 2.27–2.56 (lettuce), and 2.55–2.76 (watercress) cfu/100 ml.

Figure 6 presents the $\log_{10}$ pathogenic bacteria counts in samples collected from the four farming sites. The highest counts of *Salmonella* sp. and *E. coli* O157:H7 were observed in canal water samples, followed by soil samples, lettuce samples, and lastly, watercress samples, which showed the lowest counts. Specifically, the $\log_{10}$ counts of *Salmonella* sp. ranged from 3.8–3.9, 3.2–3.4, 1.3–1.4, and 1.4–1.5 cfu/100 ml for canal water, soil samples, lettuce samples, and watercress samples, respectively. Conversely, the log10 counts of *E. coli* O157:H7 were smaller, ranging from 2.63–2.76, 2.31–2.36, 1.02–1.17, and 0.77–0.98 cfu/100 ml for canal water, soil samples, lettuce samples, and watercress samples, respectively.

Survival of bacterial indicators and pathogens

The microbial challenge test was carried out to study the growth and survival of *Salmonella* sp., *E. coli*, and *E. coli* O157:H7 on watercress and lettuce samples. Figure 7 presents the survival of *Salmonella* sp., *E. coli* O157:H7, and *E. coli* in lettuce samples after inoculation, with bacterial load estimated every 24 hours for 15 days. The initial bacterial load of *Salmonella* sp., *E. coli* O157:H7, and *E. coli* in watercress samples was 4 log10 cfu/g.

The bacterial loads increased during the first 2–4 days of the challenge test before any meaningful decrease was noticed. *E. coli* showed the highest count increase for 4 days after incubation, followed by *Salmonella* sp. (3 days) and *E. coli* O157:H7 (2 days). Subsequently, the bacterial growth was significantly reduced from the initial counts. A reduction of 2 logs was observed in *E. coli* and *Salmonella* sp. counts after 10 and 13 days of inoculation, respectively. The reduction in counts of *E. coli* O157:H7 was 3 logs after 12 days of inoculation.

Figure 8 summarizes the survival of *Salmonella* sp., *E. coli* O157:H7, and *E. coli* in watercress samples after inoculation. Similar to the results of the challenge test on lettuce samples (Figure 7), an increase in bacterial load was observed in the first few days after inoculation, followed by a significant decrease in bacterial counts. *E.*

coli exhibited the highest increase in counts for 4 days after incubation, followed by *Salmonella* sp. (3 days) and *E. coli* O157:H7 (2 days). A reduction of 3 logs was observed after 14 days of inoculation for both *E. coli* and *E. coli* O157:H7, while the reduction in counts for *Salmonella* sp. was observed after 15 days.

Effect of washing strategies

Table 2 summarizes the effect of four different washing methods (W1, W2, W3, and W4) on reducing *E. coli*, *Salmonella* sp., and *E. coli* O157:H7 counts in lettuce and watercress samples. Regardless of the washing method, the counts of *E. coli*, *Salmonella* sp., and *E. coli* O157:H7 were reduced by 1.23–2.63, 1.24–2.58, and 0.94–2.25 $\log_{10}$ units, respectively, in lettuce samples, and by 1.4–2.7, 1.23–2.42, and 1.01–2.19 $\log_{10}$ units, respectively, in watercress samples.

All washing methods substantially reduced *E. coli*, *Salmonella* sp., and *E. coli* O157:H7 compared to the unwashed control (W0). Furthermore, vinegar-based washing methods were more effective in reducing the counts of *E. coli*, *Salmonella* sp., and *E. coli* O157:H7 than tap water-based methods, with or without salt. Combining vinegar and salt after tap washing (w4 method) had the strongest effect on reducing the counts of *E. coli*, *Salmonella* sp., and *E. coli* O157:H7 in both lettuce and watercress samples.

This study demonstrates that the microbiological quality of all irrigation water samples, regardless of the farming sites, was poor. Several reasons, such as using raw wastewater and inputting untreated wastewater into the irrigation canals, may have caused the contamination of irrigation water with high levels of fecal coliforms and pathogenic bacteria in the studied farming sites (Woldetsadik *et al.,* 2017).

High levels of fecal bacterial indicators (fecal coliforms, *E. coli*, and *Enterococci*) and pathogenic bacteria (*Salmonella* sp. and *E. coli* O157:H7) were found in both irrigation water (from the Bahr El-Baqar drain and the irrigation canal) and the farming soil (Figures 3–6). These results indicate that using water from the Bahr El-Baqar drain and/or the irrigation canals, which are polluted with untreated sewage, is not suitable for irrigation purposes, particularly for ready-to-eat crops and vegetables, according to the Food and Agriculture Organization (FAO) standard guidelines (Elkorashey, 2022).

The main source of fecal pollution appears to be untreated and/or partially treated sewage originating from households. This finding is consistent with the results of studies conducted by Schreiber *et al.,* (2015), which demonstrated that anthropogenic inputs have a substantial impact on the microbiological quality of surface water.

The watercress and lettuce samples collected from all farmlands were found to have high levels of fecal bacterial indicators and pathogenic bacteria that exceeded recommended thresholds, which indicates the poor quality of irrigation water (Amoah *et al.,* 2007a).

The presence of high levels of fecal bacterial indicators and pathogenic bacteria may be due to small streams and canals that provide low dilution, as noted by Woldetsadik *et al.,* (2017).

Unfortunately, in Egypt, farmers and traders wash ready-to-eat vegetables, including watercress and lettuce, with irrigation water from canals to remove soil and clay residues and keep them fresh for consumers. However, this practice may put consumers at risk since watercress and lettuce used in kitchen salads are not subjected to heat treatment. A similar method has been reported in Ghana and must be addressed along with other potential post-harvest contamination issues (Amoah *et al.,* 2011).

Bacterial challenge tests are conducted on food products to simulate the growth and survival of bacteria at various stages of the food manufacturing process, including preparation, processing, handling, and distribution. Such tests can determine the ability of bacteria to utilize the food as a substrate, thus indicating the risk of food spoilage or potential health hazards. Precise knowledge of how to conduct and interpret the results of challenge tests can assist food manufacturing facilities in verifying that their products meet the necessary microbiological requirements for quality and safety (Noor *et al.,* 2015).

Cordano and Jacquet (2009) and Rahman and Noor (2012) reported that the earlier detection of large numbers of microorganisms (from 10^5 to 10^8 cfu/g) in raw and ready-to-eat vegetables increases the potential for those vegetables to serve as a substrate for the growth and proliferation of spoilage microorganisms. Consequently, the growing consumption of raw and ready-to-eat vegetables has led to a need to study how the microbial environment of these items affects produce safety.

Table.1 Physicochemical characterization of Bahr El-Baqar and irrigation canal samples.

Parameters	Unit	Average (Mean ± SD)	
		Bahr El-Baqar (BB)	Irrigation Canal (IC)
pH	-	7.21 ± 0.15	7.4 ± 0.32
Temp	°C	27.4 ± 1.01	28.1 ± 1.3
TDS	mg/l	688 ± 142	310 ± 128
TSS	mg/l	148 ± 32	108 ± 18
COD	mg/l	26 ± 11	24 ± 8
BOD_5	mgO_2/l	20 ± 9	18 ± 7
TN	mg/l	11.28 ± 4.5	10.76 ± 3.8
O&G	mg/l	12.2 ± 2.9	10.1 ± 3.6

Table.2 Effect of different washing strategies on the reduction of *E. coli*, *Salmonella* sp., and *E. coli* O157:H7 counts of lettuce and watercress samples (n = 3 for each method).

Bacteria	Washing strategy*				
	W0	W1	W2	W3	W4
Lettuce (log_{10} average cfu/100 ml)					
E. coli	4.85	3.62	3.21	2.66	2.22
Salmonella sp.	4.68	3.44	3.18	2.25	2.10
***E. coli* O157:H7**	4.32	3.38	3.11	2.22	2.07
Watercress (l og_{10} average cfu/100 ml)					
E. coli	4.83	3.43	3.18	2.46	2.13
Salmonella sp.	4.55	3.32	3.14	2.21	2.13
***E. coli* O157:H7**	4.28	3.27	3.07	2.12	2.09

*W0: unwashed (control), W1: washing with tap water and rinsing for 2 min, W2: washing with tap water and rinsing for 2 min then submerged in salt solution (30 ppm) for 1 min; W3: washing with tap water and rinsing for 2 min then submerged in a vinegar solution (12000 ppm) for 1 min; and W4: washing with tap water and rinsing for 2 min then submerged in a combined solution of vinegar (12000 ppm) and salt (30 ppm) for 1 min.

Figure.1 Bahr El-Baqar drain system in the study area.

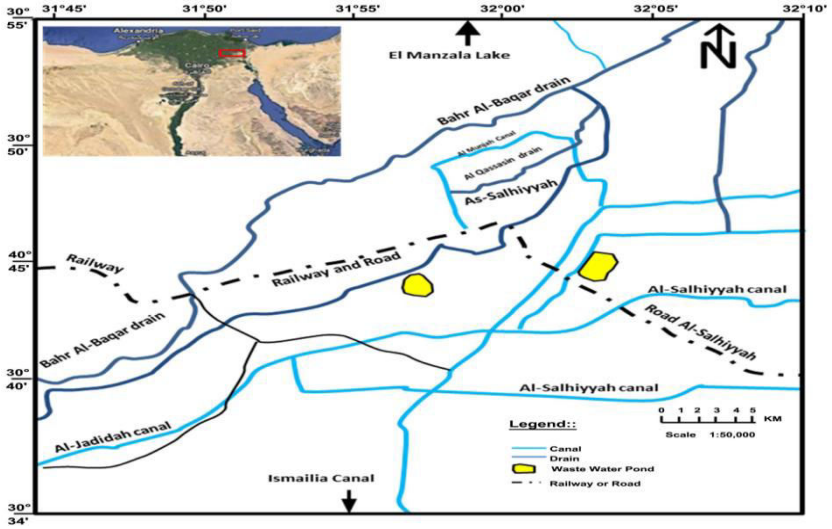


Figure.2 Illegal discharge of untreated wastewater into irrigation canals.

Figure.3 Fecal Bacterial indicators of Bahr El-Baqar samples (set I) from the four farmlands (F1, F2, F3, and F4); B: Bahr El-Baqar, S: soil, W: watercress, L: lettuce.

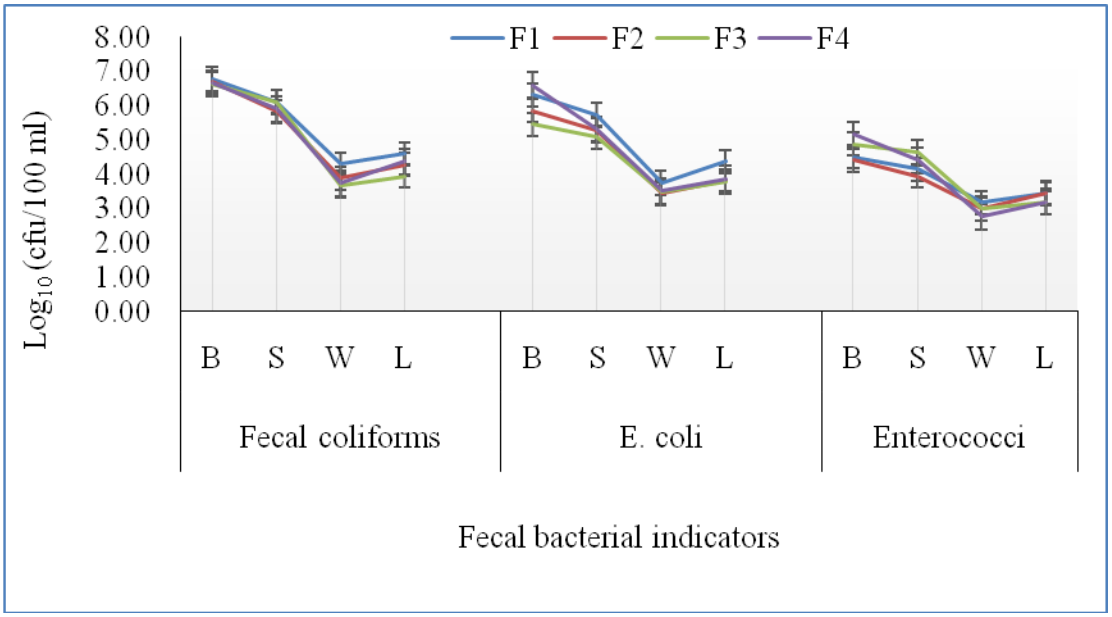


Figure.4 Pathogenic bacteria of Bahr El-Baqar samples (set I) from the four farmlands (F1, F2, F3, and F4); B: Bahr El-Baqar, S: soil, W: watercress, L: lettuce.

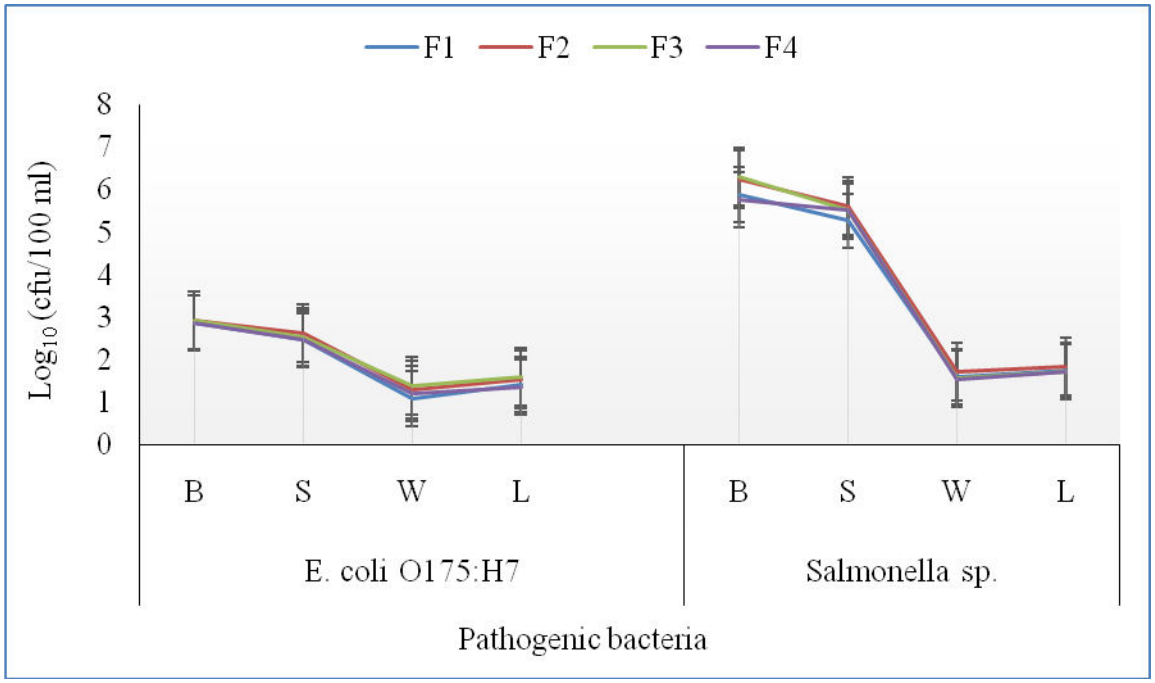


Figure.5 Fecal Bacterial indicators of canal water samples (set II) from the four farmlands (F1, F2, F3, and F4); C: canal water, S: soil, W: watercress, L: lettuce.

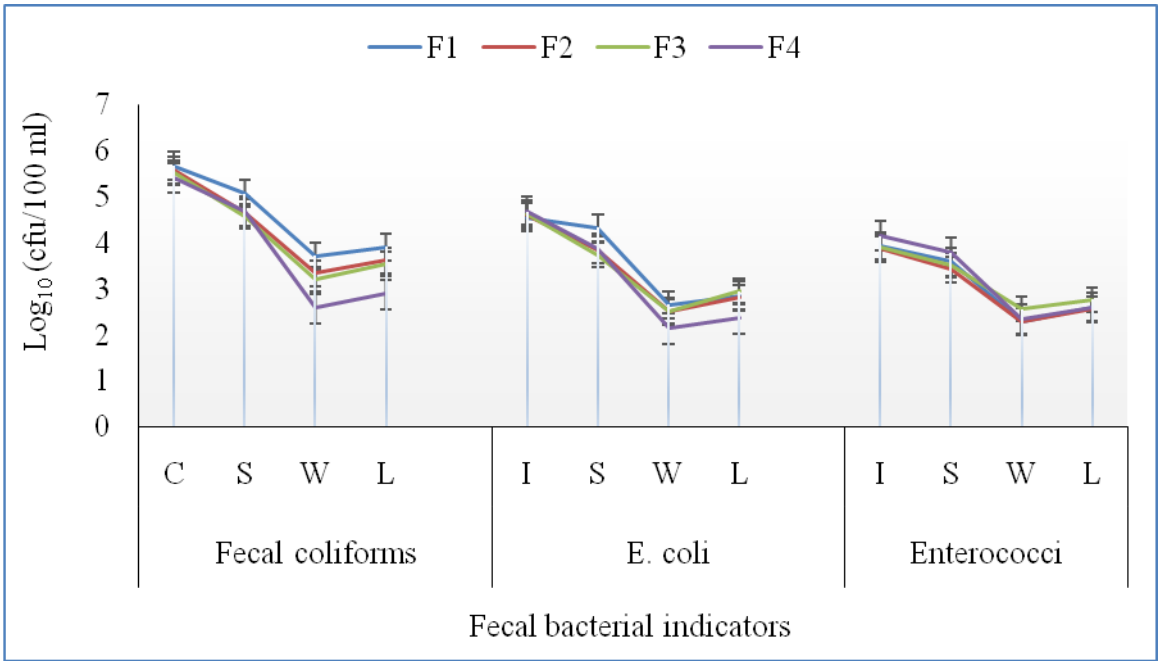


Figure.6 Pathogenic bacteria of canal water samples (set II) from the four farmlands (F1, F2, F3, and F4); C: canal water, S: soil, W: watercress, L: lettuce.

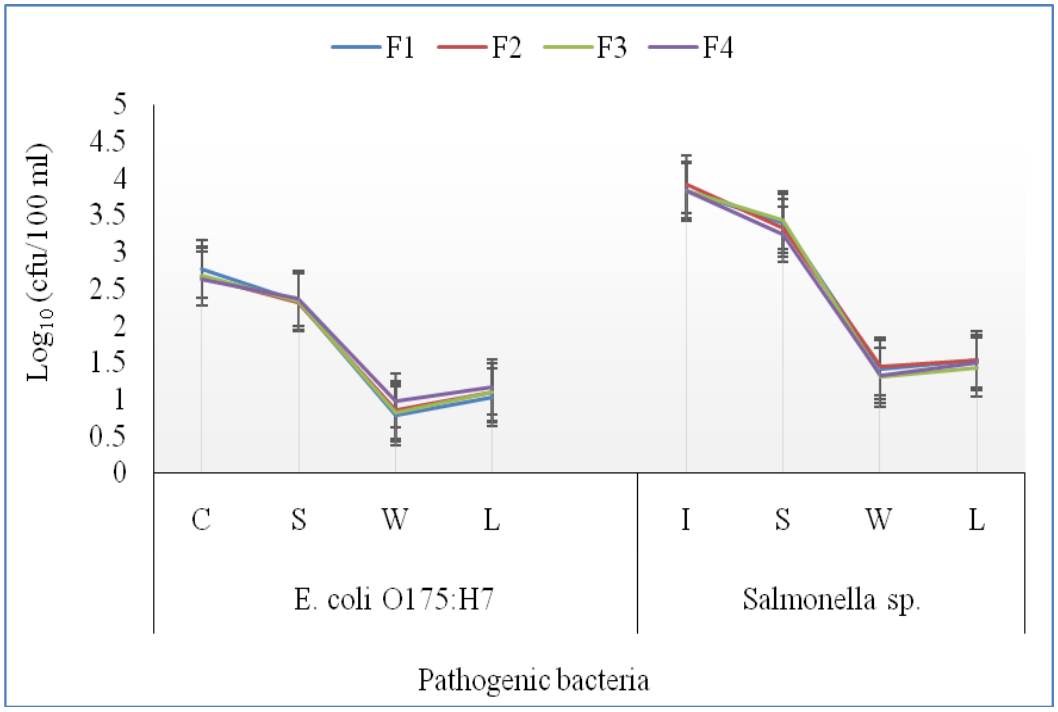


Figure.7 Survival of Salmonella sp., *E. coli* O157:H7, and *E. coli* in lettuce samples.

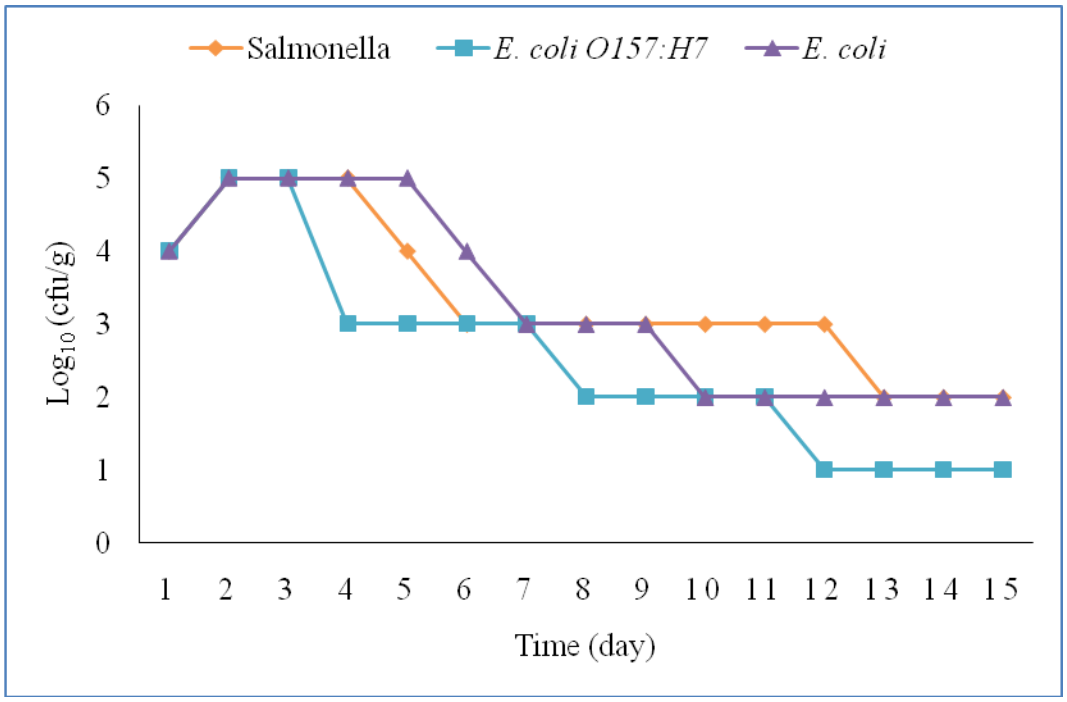
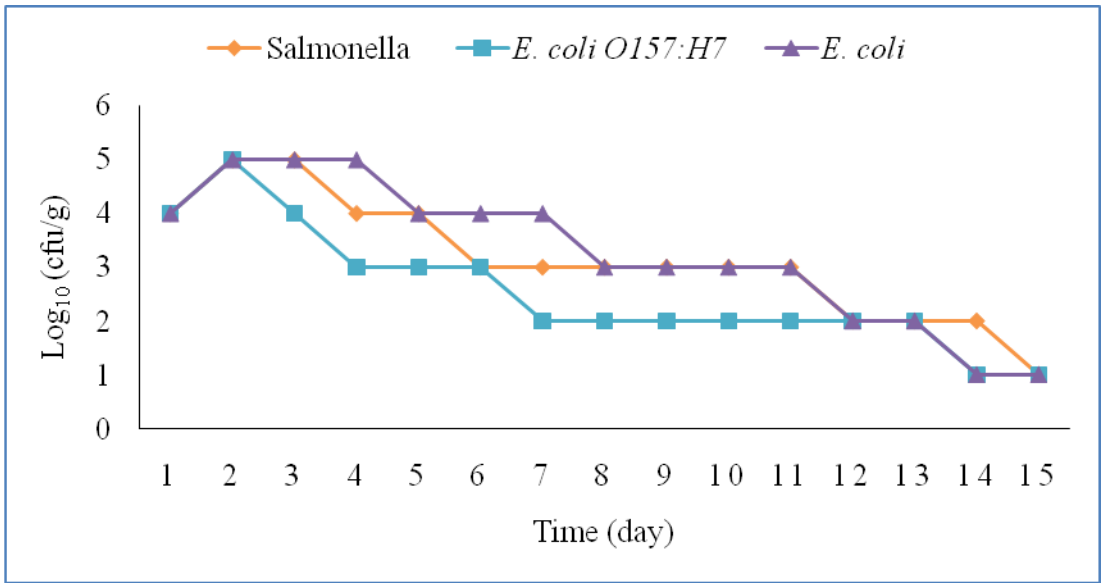


Figure.8 Survival of Salmonella sp., *E. coli* O157:H7, and *E. coli* in watercress samples.



The results obtained during this study indicated that *Salmonella* sp., *E. coli*, and *E. coli* O157:H7 survived with high numbers on watercress and lettuce samples (Figures 7 and 8). Many fecal bacterial indicators and pathogenic bacteria suggested that fresh watercress and lettuce were suitable substrates for microorganisms.

The bacterial culturable cells steadily increased after inoculating watercress and lettuce samples, indicating that fresh vegetable supplies can promote bacterial growth. However, the subsequent decline in bacterial growth may be caused by nutrient depletion or malfunctioning bacterial genes (Fatema *et al.,* 2013; Feroz *et al.,* 2013). Noor *et al.,* (2015) reported similar findings after inoculating different bacterial species, such as *E. coli*, *Salmonella*, *Klebsiella*, *Pseudomonas*, *Bacillus*, *Listeria*, *Staphylococcus*, and *Vibrio*, on various vegetables, including carrots, tomatoes, cucumbers, and lettuce. The final decrease in bacterial burden suggested that the tested bacterial population had utilized the substrates to their limiting concentrations. The microbial challenge test can identify several factors that influence the growth and survival of microbial pathogens on the surface of ready-to-eat vegetables, including the type of vegetable and inoculum size (Feroz *et al.,* 2013; Noor *et al.,* 2015). Understanding how fecal bacterial indicators and pathogenic bacteria survive on fresh vegetables may aid in determining food safety, food stability, and, ultimately, consumer safety. The practical use of the bacterial challenge test relies on maintaining specific microbiological procedures during product processing and handling to ensure a longer product life. This study focuses on potential bacteriological hazards for consumers and washing techniques to reduce fecal bacterial indicators and pathogenic bacteria instead of examining farmer exposure to wastewater, which has been addressed in previous studies and for which universally recognized risk mitigation strategies exist (WHO, 2006). Our results (Table 2) indicate that all examined washing methods were effective in reducing fecal coliform and pathogenic bacteria levels, but the vinegar-based techniques demonstrated the greatest reduction. Two vinegar-based washing methods were used in our study, one without salt (W3) and the other with a small amount of salt (W4), and there was a slight difference in their effectiveness in removing fecal coliform and pathogenic bacteria.

Therefore, it is important for households to understand that using a small amount of salt may have benefits, as observed by Woldetsadik *et al.,* (2017). Longer contact times and high sanitizer concentrations, as reported by Amoah *et al.,* (2007b), were effective in significantly reducing fecal coliform levels, but given the additional processing time, expense, and impact on lettuce quality, the viability of increasing contact times and concentrations above certain levels was questioned.

Additionally, household bleach (sodium hypochlorite) can be used effectively for food sanitation. The effectiveness of the technique employed to reduce the microbial population may also be influenced by

additional factors, such as the type and physiology of the target organisms and the features of product surfaces (Parish *et al.,* 2003). The level of bacterial indicators and/or pathogenic bacteria on ready-to-eat vegetables is also affected by the style of washing (running vs immersion), which has implications for the final risk assessment. For example, according to Pangloli *et al.,* (2009), using tap water for washing is more effective than immersion and can reduce *E. coli* O157:H7 levels by up to 2.2 $\log_{10}$.

In conclusion, the current study indicates that using water contaminated with untreated (illegal dumping of raw sewage in irrigation canals) or partially treated sewage for irrigating ready-to-eat vegetables, such as watercress and lettuce, contaminates these vegetables with high levels of fecal bacterial indicators and pathogenic bacteria. Irrigation with water contaminated with untreated or partially treated sewage increases the bacterial levels in the farming soil, increasing the bacterial load of fecal coliforms and pathogenic bacteria on ready-to-eat vegetables.

E. coli, *E. coli* O157:H7, and *Salmonella* sp. showed significant survival on watercress and lettuce for more than 15 days. The results of the current study demonstrate that levels of *E. coli* and pathogenic bacteria can be reduced using vinegar-based washing strategies, with or without the addition of salt. The World Health Organization (WHO) recommends a multi-barrier strategy, and washing green salads at home is one of the barriers. Post-harvest treatment is crucial to prevent post-harvest contamination, which can occur when harvested vegetables are washed on the farm. In addition to limiting occupational exposure, risk reduction initiatives should target households and restaurants without guidance on the best way to wash ready-to-eat vegetables.

Author Contribution

Shimaa R. Hamed: Investigation, formal analysis, writing—original draft. Raed S. Al-Wasify: Validation, methodology, writing—reviewing. Samar Ragab:—Formal analysis, writing—review and editing. Aya A. Abd-Elrahim: Investigation, writing—reviewing.

Data Availability

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

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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