SAFE PRODUCTION OF ONION – 2016, UNDERSTANDING THE FATE OF *ESCHERICHIA COLI* IN THE SOIL

Stuart Reitz, Malheur County Extension, Oregon State University, Ontario, OR

Clinton C. Shock, Erik B. G. Feibert, Alicia Rivera, and Lamont D. Saunders, Malheur Experiment Station, Oregon State University, Ontario, OR

Harry Kreeft, Western Laboratories, Parma, ID

Jim Klauzer, Clearwater Supply, Ontario, OR

Introduction

The Food Safety Modernization Act (FSMA) seeks to protect human health. The FDA has proposed rules to implement the FSMA that include regulations on limits of how much generic *Escherichia coli* will be allowed in irrigation water. Although the recently released revisions to the FSMA rules appear favorable to the onion industry, growers will still need to meet the FDA's guidelines, which mandate substantial testing of irrigation water quality. In addition, buyers must remain satisfied that onions from the Idaho-Eastern Oregon region meet their quality standards.

The FDA has expressed concern that onions irrigated with water containing *E. coli* could become contaminated with the bacteria on their surface or interior. While *E. coli* is ubiquitous in the environment, specialized strains of *E. coli* can cause human health problems including dehydration, gastroenteritis, kidney failure, and other conditions. The presence of human pathogenic *E. coli* on raw and processed foods has been linked with various outbreaks of illness in the United States. However, illnesses have not been linked to the production or shipping of onions.

The FDA is expected to issue further guidance on water testing procedures in the near future. The current project continues to examine key production factors related to onion irrigation to address the FSMA rules so that onions can continue to be produced safely, at low risk, and at competitive cost with national and international competitors.

In previous trials, we have demonstrated that regardless of *E. coli* levels in irrigation water, only a small fraction of the *E. coli* is delivered to the soil immediately adjacent to onion bulbs in the field (Shock et al. 2015; Shock et al. 2016a,b) This finding applies to both furrow and subsurface drip irrigation. The silt loam soil at the Malheur Experiment Station retained most of the *E. coli* away from the onion bulbs and close to where the water entered the soil. In these trials, no *E. coli* was detected inside of the onion bulbs from any irrigation treatment. Sporadic occurrences of *E. coli* were found on bulb exteriors; however, these cases appear to be from random environmental contamination unrelated to irrigation water quality. Based on these findings, current subsurface drip or furrow (flood) irrigation practices do not appear to pose a significant risk for bacterial contamination of dry bulb onion grown on silt loam. However, these previous trials have analyzed only *E. coli* levels in the soil at one time point following an irrigation event. The fate of

E. coli over time following irrigation has not yet been studied to determine how rapidly it may die-off in the soil.

Our objectives were to determine the impact of contaminated irrigation water on the relative safety of dry bulb onions:

a. Evaluate survival/distribution of generic *E.coli* in the soil over time following irrigation with *E. coli* contaminated water.

b. Compare levels of *E. coli* in sterilized and unsterilized soil to determine if the presence of naturally occurring soil microbes affects survival of *E. coli*.

c. Determine if the presence of onions affects levels of *E. coli* in the soil compared to soil where no plants were growing.

The public will benefit from the results of this study by being assured that onion bulbs are produced safely. Growers will benefit by the assurance of market access and minimal additional imposition of added costs of production. Growers and companies that store and ship fresh market onions will have tools available to make sure that they are delivering products with low health risk to the public.

Materials and Methods

The trial was conducted at the OSU Malheur Experiment Station using 'Vaquero' onions and conventional production practices. We used a randomized, replicated experimental design, with large sample sizes to provide statistical power.

To determine if the presence of onions affected *E. coli* in the soil, we established plots with onions and bare-ground plots with no onions or other vegetation. There were five replications of the onion and bare-ground treatments.

The onion treatment plots were planted with 'Vaquero' onions on March 24, 2016 at 150,000 seeds/acre. The trial was irrigated exclusively by subsurface drip irrigation, using well water until August 23. Two double rows of onions were planted on 44-inch beds. Bare ground plots received the same irrigation as the onion plots.

Drip tape was laid at planting at 3-inch depth in the middle of the bed serving two double rows of onions, one pair to each side of the tape. The drip tape had emitters spaced 12 inches apart and emitter flow rate of 0.13 gal/hour (Toro Aqua-Traxx, Toro Co., El Cajon, CA). The distance between the tape and the center of each double row of onions was 11 inches. Until the experiment began, all onions were irrigated automatically with well water to maintain the soil water tension (SWT) in the onion root zone below 20 kPa (Shock et al. 2013). Soil water tension was measured with granular matrix sensors (GMS, Watermark Soil Moisture Sensors Model 200SS, Irrometer Co., Inc., Riverside, CA) installed at an 8-inch depth in the center of the double row. Sensors had been calibrated to SWT.

Furrow irrigation, which delivers more water to the field and hence more *E. coli*, was used for the final irrigation. Water from a cement canal was delivered to furrows that had been created previously in the field. Lay-flat hose (2-inch diameter) was placed to deliver water from the

canal to the furrows in the field. The lay-flat was connected to PVC pipe, and the PVC pipe was connected directly to the source canal, with water flow controlled by a plate in the canal.

To raise the *E. coli* concentration in the irrigation water, burlap bags filled with cow manure were placed in the canal upstream of the outlet. Tins placed in the canal created turbulence and accelerated flow to ensure mixing of manure into the irrigation water. The furrow irrigation system delivered approximately 0.75 gal/min/furrow.

The final irrigation, with the *E. coli*-enhanced irrigation water, was conducted on August 23, 2016. The burlap bags with manure were placed in the canal for 45 min before the irrigation began, to allow for mixing. Irrigation was then delivered to the field for 8 hours.

Water samples were collected from the canal each hour during irrigation to determine *E. coli* levels. Samples were also collected upstream of where the manure was placed in the canal at the beginning and end of the irrigation period to determine baseline levels of *E. coli* in the canal.

The day before the irrigation, sterilized soil was placed in containers that we have used in previous trials (Sterilized Soil Solution Capsules). Soil was collected from the experimental field and sterilized by baking in an oven at 180^{o}F for 4 days. Sets of these Sterilized Soil Solution Capsules were placed next to the furrow, at 2 inches from the furrow, and 2 inches from that midpoint location. In onion plots, this third location was next to onions in the outer row of the bed (Fig. 1).

Soil from the field was collected using the same spatial arrangement (i.e., next to furrow, midpoint, next to onion row; Fig. 1). Soil was sampled after an 8-hour irrigation set from furrow irrigated beds at three locations as described above. Soil was collected by using sterilized plastic putty knives to extract a wedge of soil 2 inches deep, 1 inch wide, and 4 inches long parallel to the water sources and onion rows from each sample location.

Soil samples were collected according to the schedule in Table 1. Two soil samples and two capsules were collected from each location in each plot on each sample day. One of each pair was collected from each side of a furrow (i.e., from the right and left sides of the furrow). Soil samples were collected from each plot the day before the irrigation to determine baseline levels of soil *E. coli.* A set of six sterile soil solution capsules that had not been placed in the field was also collected to determine baseline *E. coli* levels in the soil.

Two weeks after the last soil samples were collected (i.e., 3 weeks after the irrigation event), 2 samples of 30 onions each were collected from each of the onion plots to determine if *E. coli* was present on the exterior or in the interiors of the bulbs. Onions were analyzed at Western Laboratories, Inc. (Parma, ID) for *E. coli*. Roots, small remnants of soil, skins, and outer peel of the onions were removed from the bulbs and weighed, and were then thoroughly washed in 1 L of water. A 100-ml sample of the wash water was used to estimate a Most Probable Number (MPN) of generic *E. coli* present on the exteriors of the onions, using IDEXX Colilert® +Quanti-Tray/2000® (IDEXX Laboratories, Westbrook, ME) (Edberg et al. 1990). The mean *E. coli* MPN per onion bulb exterior was calculated based on the number of onions in each sample.

To test for the presence of internal *E. coli* the outsides of the peeled onions were disinfected with 70% ethanol, and then the bulbs were placed on a sterilized aluminum tray. A wedge was cut from each onion, and the wedges were placed in a sterilized stainless steel beaker and macerated with a food processer. After maceration, 10 ml of the resulting onion suspension was placed in a flask containing 90 mL of Universal Pre-enrichment broth (UPB, Accumedia, Nedgen, MI) and

sealed. Along with every batch of samples, an additional positively inoculated sample was placed in an additional flask containing UPB broth. The UPB broth was placed in an incubator for 48 hours at 35°C. A glass jar with 100 ml sterilized water had a package of Colisure (IDEXX) added as a substrate to test for the presence of generic *E. coli*. Five ml of the UPB was transferred to the Colisure mixture and incubated for 24 hours at 35°C. After 24 hours the Colisure samples were examined under UV light for fluorescence, indicating the presence of generic *E. coli* (Edberg et al. 1990).

Table 1. Sampling schedule for collection of normal soil and sterile soil solution capsules from plots with onions and with bare ground. Numbers in the columns are the total number of samples collected each day. Malheur Experiment Station, Ontario, OR, 2016.

Sample day	Normal soil sample (no.)	Sterile soil (SSSC) (no.)
Day 0 (before irrigation	60	6 (to establish soil is free of *E. coli*)
Day 1 (day of irrigation)	60	60
Day 2	60	60
Day 3	60	60
Day 4	60	60
Day 7	60	60

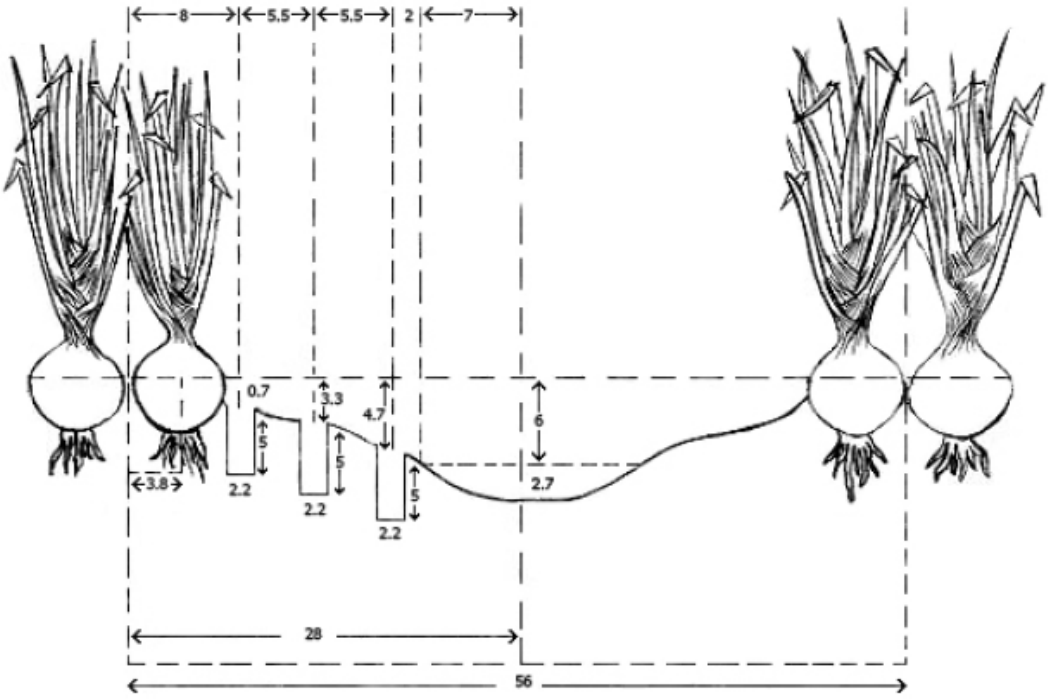

Figure 1. Location of soil samples in furrow-irrigated onions with respect to edge of the water and onion bulbs. The same spatial arrangement was used in bare ground plots. Distances are in cm. Diagram is adapted from Shock et al. 2016b.

Soil and water samples were refrigerated immediately after collection and transported to Western Laboratories for analysis. Soil samples were divided with one part weighed wet, dried, and weighed dry to determine the soil water content. The other part of each sample was diluted in water; 50 ml of the water mixture was used to estimate the MPN of generic *E. coli* in the soil water using IDEXX Colilert® + Quanti-Tray/2000® (Edberg et al. 1990).

The MPN of generic *E. coli* in the soil water was compared between the onion and bare ground treatments, and among soil sampling locations on each sample date. Changes in *E. coli* levels across dates were also analyzed. MPN data were transformed to log10 before analysis. Data were subjected to analysis of variance with treatments being the main plots, sampling positions as split plots, and days as repeated measurements over time.

Results

The silt loam soil had very low levels of *E. coli* before the irrigation event. There was no *E. coli* detected in 25 of the 30 soil samples (Fig. 2). The only baseline samples in which *E. coli* was detected were five of the samples from one of the bare-ground plots. This finding suggests there may have been some incidental contamination in that one particular plot. No *E. coli* was detected in the baseline sterile soil solution capsule samples (Fig. 2).

We were able to raise the *E. coli* levels in the canal water with the addition of cow manure. No *E. coli* was detected in the water samples collected upstream from the manure. The manure elevated *E. coli* levels to more than 242,000 MPN/100 ml of water during the irrigation cycle.

Based on previous trials, *E. coli* levels from soil next to onion bulbs are dramatically lower than at locations closer to the water source (i.e., closer to a furrow or closer to a drip tape). To determine if *E. coli* levels are affected by the presence of onions versus simply a function of the distance water travels through the soil, we sampled corresponding locations in beds where no onions are planted. We found no statistical difference between *E. coli* levels from plots with onions versus plots with bare ground. This result was true for field-collected soil and for sterile soil solution capsules (Figs. 3 and 4). All soil samples were collected from areas where irrigation water had not yet passed beyond onions in the bed, which may have reduced any potential antimicrobial influence of the onions. However, it appears soil properties or soil-dwelling organisms are more responsible for reducing *E. coli* levels than is the presence of onions.

The silt loam soil from plots with onions sampled immediately after irrigation had very high levels of *E. coli* when collected from locations alongside the irrigation furrow. However, levels declined dramatically with increasing distance from the furrow (Figs. 3 and 4). These results are similar to results from previous trials that we have conducted. Soil samples from the bare-ground plots showed similar patterns as those from the onion plots (Fig. 5).

Twenty-four hours after the conclusion of irrigation, *E. coli* levels in the onion plots had decreased by several orders of magnitude, and levels continued to decline significantly over the remaining sample days. The same trend of *E. coli* levels decreasing with greater distance from the furrow occurred over time.

For the sterile soil capsules, initial *E. coli* levels were highest in the location next to the furrow and declined with distance from the furrow. *E. coli* levels declined over time, similar to the field soil samples. In general, *E. coli* levels over sample days remained lower the farther from the furrow that they were collected (Fig. 4).

Of the 10 samples of onions collected after the last soil collection (3 weeks after irrigation), 9 had no detectable *E. coli* on the exteriors. One sample had an average of 20 MPN of *E. coli* per bulb. Because the bulbs were pooled for analysis, we could not determine the number of bulbs that actually had *E. coli.* These bulbs had not been prepared for pack-out. That process may have removed residual *E. coli* on the exterior skins, roots or soil associated with the bulbs (Reitz et al. 2016). No *E. coli* was detected in the interiors of any bulbs.

These patterns suggest that silt loam soil filters *E. coli* from the irrigation water. Given that the sterile soil capsules had higher levels of *E. coli* than the field soil on the day after irrigation, it is likely that naturally occurring soil microbes kill *E. coli* as it moves through the soil.

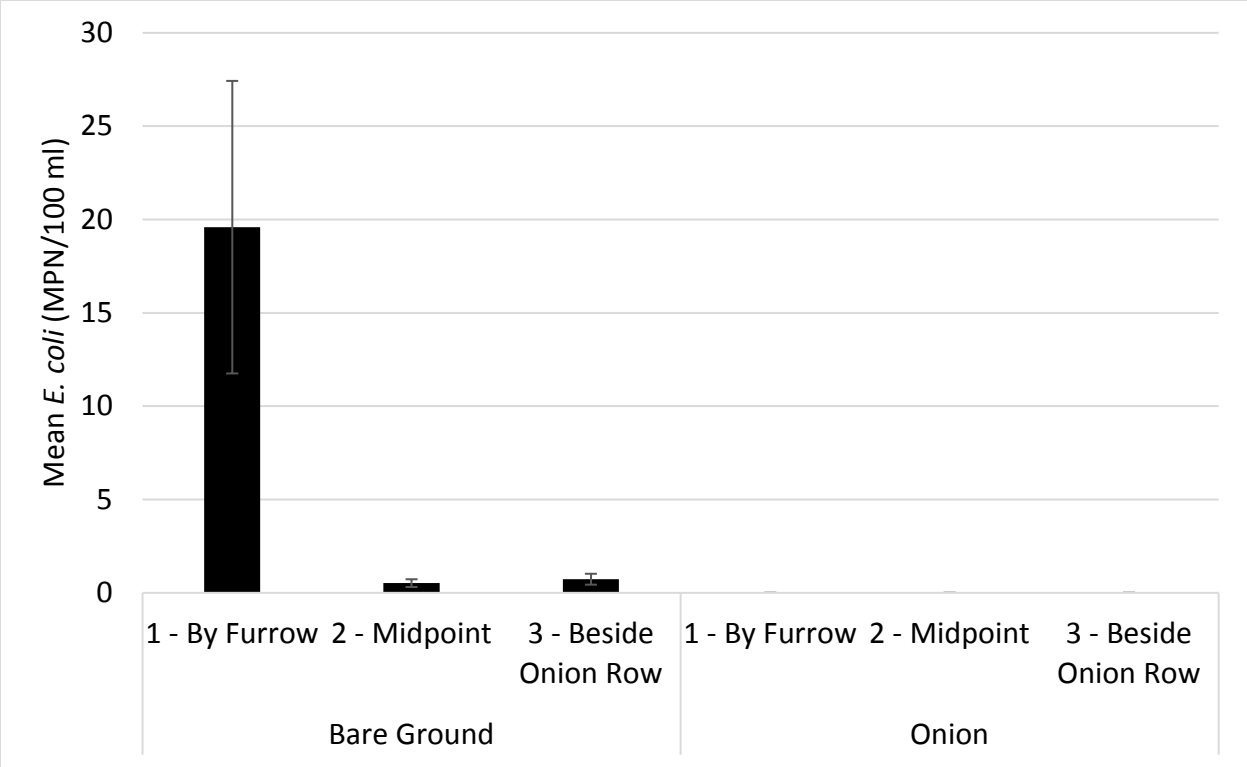

Figure 2. Baseline levels of E. coli *in soil water before final irrigation with highly contaminated water. Only 5 of 30 samples had detectable levels of* E. coli. *Those five samples came from one plot. For the onion treatment, location 1 is closest to the water source (irrigation furrow); location 2 is intermediate distance from the furrow to the outside row of onions; location 3 is next to the outside row of onions in the bed. For the bare-ground treatment, locations are the same distances from the furrow as in the onion treatment. Malheur Experiment Station, Ontario, OR, 2016.*

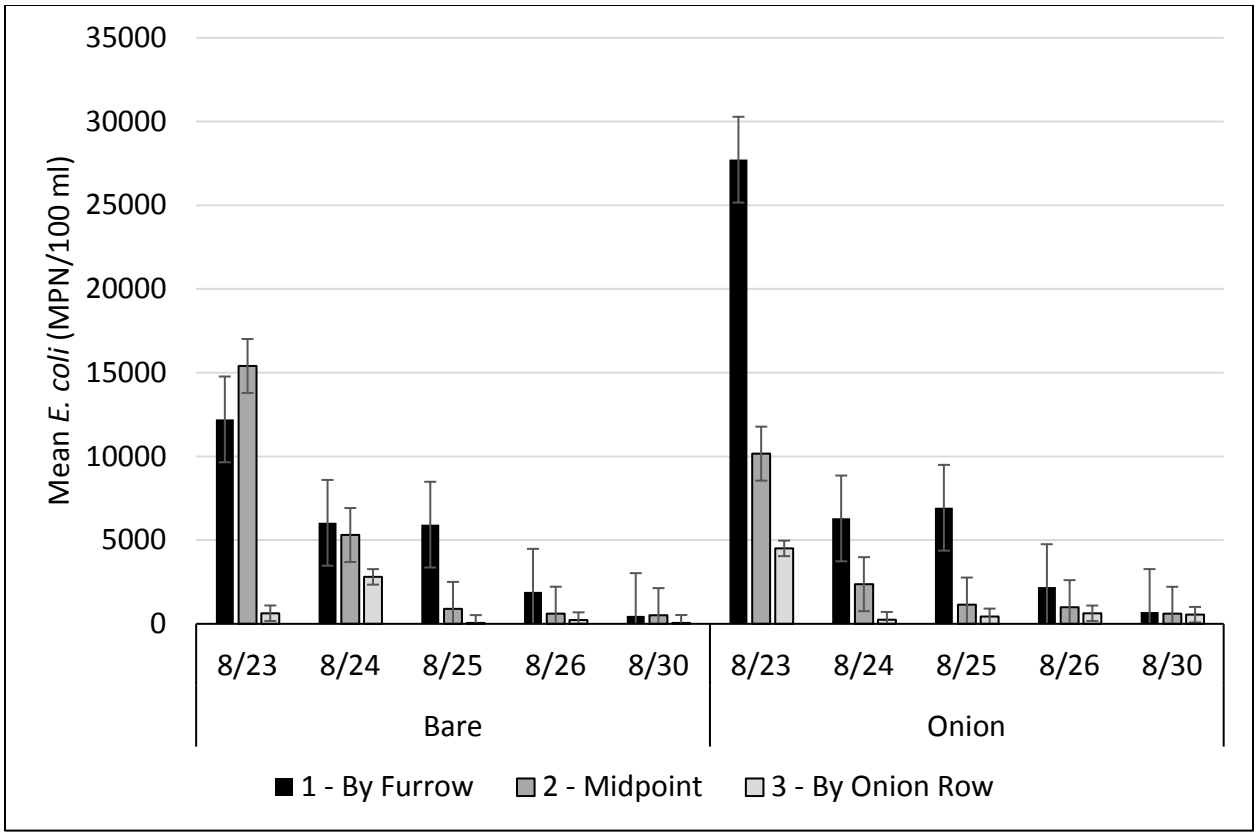

Figure 3. E. coli *levels from soil collected immediately after irrigation with highly contaminated water (on August 23, 2016) and the days following the irrigation. For the onion treatment, location 1 is closest to the water source (irrigation furrow); location 2 is intermediate distance from the furrow to the outside row of onions; location 3 is next to the outside row of onions in the bed. For the bare-ground treatment, locations are the same distances from the furrow as in the onion treatment. Malheur Experiment Station, Ontario, OR, 2016.*

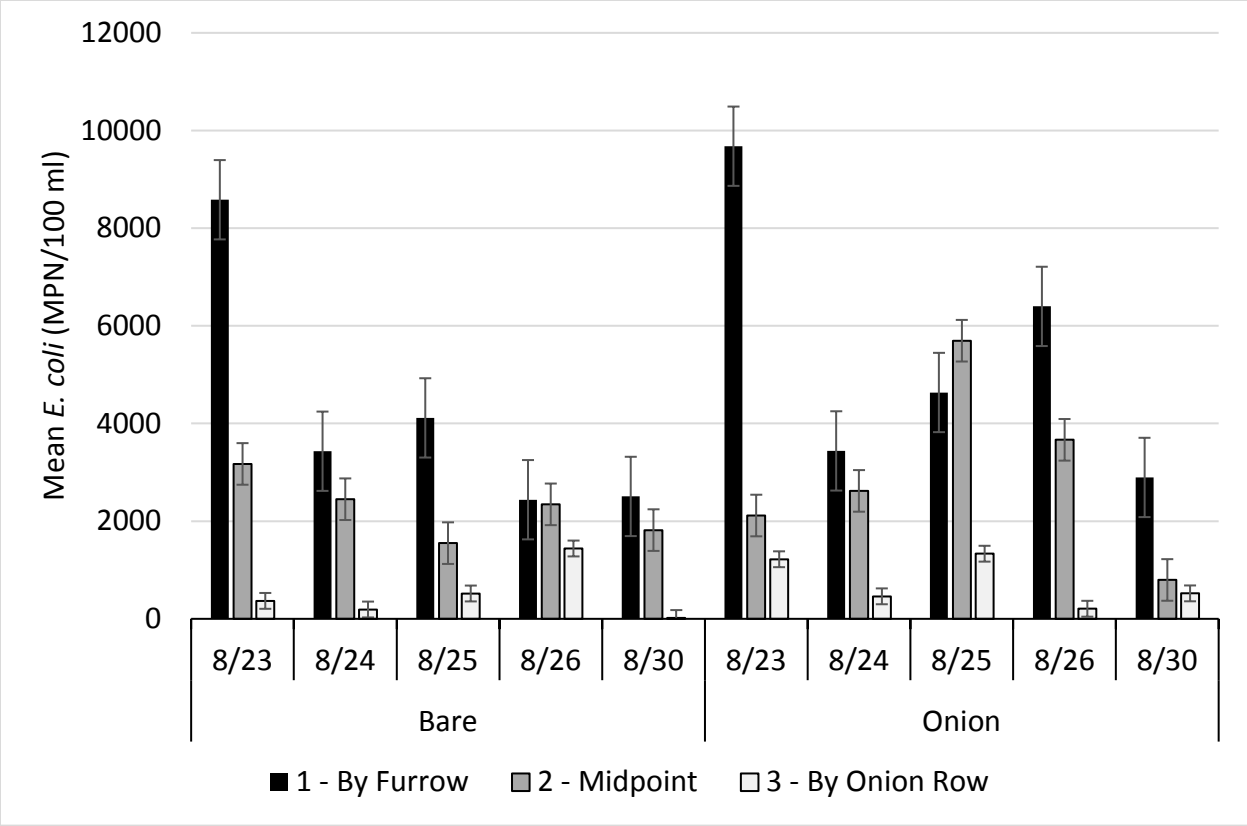

Figure 4. E. coli levels in sterile soil capsules collected immediately after irrigation with highly contaminated water (on August 23, 2016) and the days following the irrigation. For the onion treatment, location 1 is closest to the water source (irrigation furrow); location 2 is intermediate distance from the furrow to the outside row of onions; location 3 is next to the outside row of onions in the bed. For the bare ground treatment, locations are the same distances from the furrow as in the onion treatment. Capsules were placed in the ground before the irrigation event. Malheur Experiment Station, Ontario, OR, 2016.

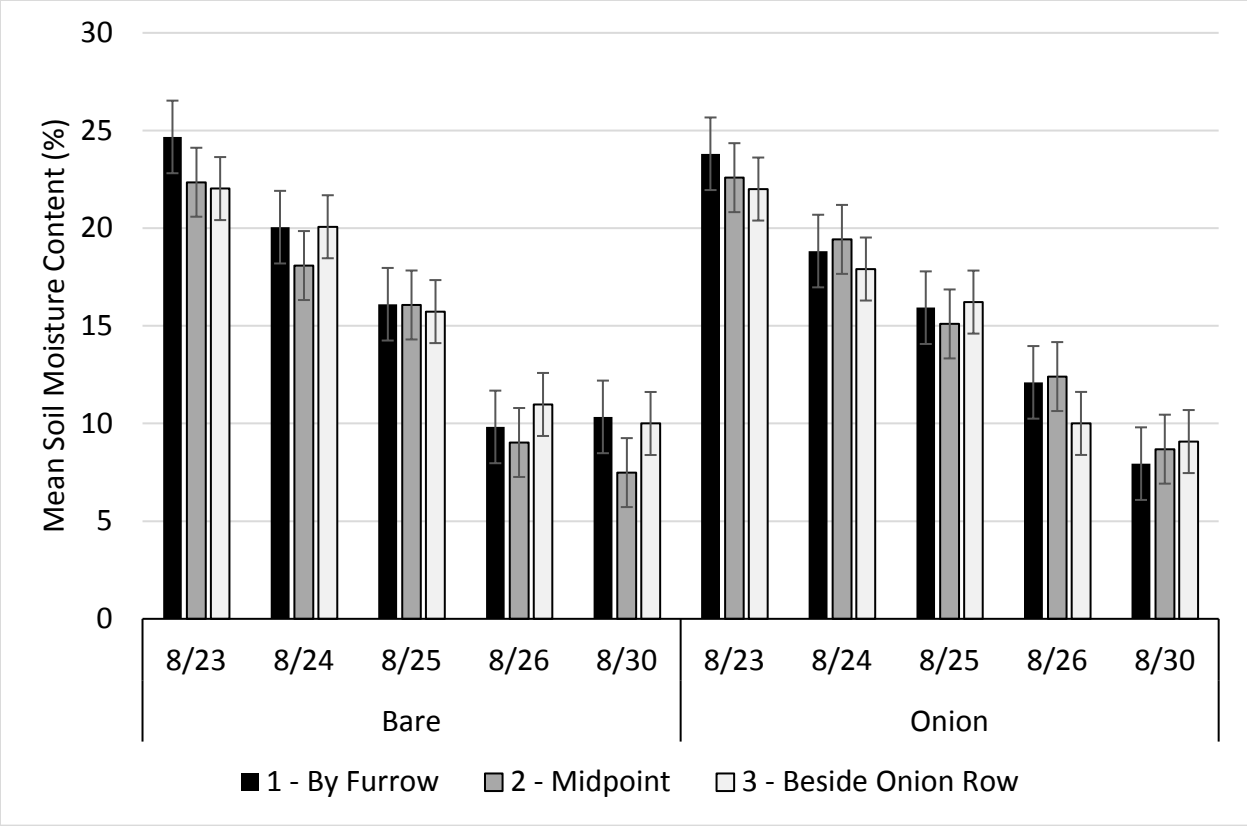

Figure 5. Changes in soil moisture content following 8-hour furrow irrigation on August 23, 2016. For the onion treatment, location 1 is closest to the water source (irrigation furrow); location 2 is intermediate distance from the furrow to the outside row of onions; location 3 is next to the outside row of onions in the bed. For the bare ground treatment, locations are the same distances from the furrow as in the onion treatment. Malheur Experiment Station, Ontario, OR, 2016.

Conclusions

The silt loam soil tended to filter *E. coli*, as levels of the bacterium were significantly higher close to where the water entered the soil than further into onion beds. Nevertheless, only a small fraction of the *E. coli* from the irrigation water was delivered to the soil water. *E. coli* levels declined significantly over 7 days from the irrigation event to the end of the sample period. These declines suggest that soil-dwelling organisms or characteristics of the soil have significant antimicrobial activity. No *E. coli* was detected inside of the onion bulbs from any irrigation treatment. Microbial quality of irrigation water does not appear to pose a significant risk for bacterial contamination of dry bulb onion grown on silt loam.

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