

EFFECTS OF LATERAL DEPTH AND WATER APPLIED ON TRANSPORT OF E. COLI IN SOIL AND RESIDUALS WITHIN PLANTS AND ASPARAGUS LETTUCE PRODUCTION FOR DRIP IRRIGATION APPLYING SECONDARY SEWAGE EFFLUENT

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ABSTRACT

Abstract. This study aims at investigating the effects of lateral depth and water applied on E. coli transport and plant growth while applying secondary sewage effluent during the growing season of asparagus lettuce in greenhouse. The experiments were conducted with three lateral depths of 0 (D0), 10 (D1) and 20 cm (D2) below the soil surface along with three irrigation levels that were determined by pan coefficient of 0.6 (I1), 0.8 (I2) and 1.0 (I3). The results indicated that the fate of E. coli in the soil was influenced by the lateral depth and the elapsed time after irrigation ceased. Generally, the population of E. coli demonstrated a decreasing trend with the growing of asparagus lettuce. Specifically, for all treatments of D0, E. coli in surface 10 cm soil decreased from 50-60 CFU/g to less than 9 CFU/g in 20 days after irrigation ceased. Only 1.0 and 0.4 CFU/g of E. coli was detected on the leave surface of plants. For all treatments, no E. coli was detected on the surface of stems. The yield ranged from 42 to 51 t/ha and the subsurface drip irrigation treatments irrigated at a pan coefficient of 0.8 produced a higher yield.

Keywords: E. coli; asparagus lettuce; lateral depth; pan coefficient; subsurface drip irrigation

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1. Introduction

Water scarcity and droughts are emerging as major issues worldwide, not only in dry lands, but also in the regions where freshwater is abundant (Bixio et al., 2006; FAO, 2012). However, the reuse of wastewater involves both health and environmental risks. Bacteria, including pathogenic *Escherichia coli* (*E. coli*), *Salmonella enterica*, *Shigella*, *Staphylococcus*, and fungal in irrigation water may stay in soil or on the surface of crop plants, transmit to people and cause disease (Crook, 1998). *E. coli* are one of the most common pathogenic bacteria that cause diseases in human. Due to its simple detection and high numbers in agricultural wastes, *E. coli* are consistently used as an indicator microorganism for the risk assessment of microbial contamination (Foppen and Schijven, 2006).

Irrigation regime influenced bacterial transportation. Oliveira et al. (2012) have proved that *E. coli* O157:H7 survived in soil and lettuce leaves while applying surface and sprinkler irrigation with contaminated water. Subsurface drip irrigation has been proved in reducing the risk of bacterial contamination compared to furrow irrigation and sprinkler irrigation (Fonseca et al., 2011). Drip irrigation is therefore often preferred in effluent irrigation systems as it can avoid direct contact between wastewater and plants, thus reducing health and environmental risks (Campos et al., 2000). Irrigation regime also influenced crop production. It is recognized that drip irrigation systems can deliver water and chemicals to the root zone of plants more efficiently than most other forms of irrigation system. Ayars et al. (1999) reviewed the subsurface drip irrigation research conducted at the USDA Water Management Research Laboratory over a period of 15 years. Their results demonstrated significant increases in yield and water use efficiency increases for all crops.

The objective of this research was to study the effects of the drip irrigation regimes on *E. coli* distribution in soil and asparagus lettuce when applying secondary effluent irrigation during the growth period. We also compare the production of asparagus lettuce and give recommendations for management of drip irrigation systems.

2. Materials and methods

2.1 Experimental design

Field experiments were conducted in a solar heated greenhouse located at the Experimental Station of the National Center for Efficient Irrigation Engineering and Technology Research in Beijing (39° 39' N, 116° 15' E, and 40.1 m above sea level). The greenhouse was 50 m in length and 8 m wide. The soil was sandy loam (Fluvents, Entisols) with a bulk density of 1.44 g/cm³, a field capacity of 0.33 cm³/cm³ and a permanent wilting point of 0.15 cm³/cm³ that was measured at 1.5 MPa suction using a centrifugal method. The region has a warm and semi-humid continental monsoon climate with an annual mean temperature of 11.6 °C and an annual mean precipitation of 556 mm.

Two factors of lateral depth and irrigation level were considered in the experiments. Secondary sewage effluent was used as irrigation water. Three lateral depths of 0 cm (D0), 10 cm (D1), and 20 cm (D2) with three irrigation levels that were determined by pan coefficient of 0.6 (I1), 0.8 (I2) and 1.0 (I3) multiplying by the evaporation rate of a 20-cm pan located at the top of the asparagus lettuce canopy. Additionally, water quality experimental treatments with three lateral depth of 0 (D0), 10 (D1), and 20 cm (D2) irrigated at pan coefficient of 0.8 (I2) with groundwater were also created (shown by letter C added). Three replicates were used for each treatment, and a total of 36 plots were arranged randomly in the greenhouse.

Each plot was 2.7 m in length and 2.1 m wide. Three driplines were installed along the median of two adjacent asparagus lettuce rows, and the two lettuce rows were irrigated by one dripline. The dripline length was 3 m and the spacing between two adjacent driplines was 0.7 m. The SDI dripline (TECHNET, Netafim Ltd., Israel) with emitters spaced at 30 cm was selected and the nominal discharge rate for an individual emitter was 1.6 L/h. In each plot, 9 asparagus lettuces were planted in one row, and were transplanted at a row spacing of 35 cm and a plant spacing of 30 cm along a row on 28 August 2014. The asparagus lettuces were harvested on 31 October 2014, and the yield was measured by weighting 16 asparagus lettuces samples shown in Fig. 1.

2.2 Sample collection and enumeration of *E. coli*

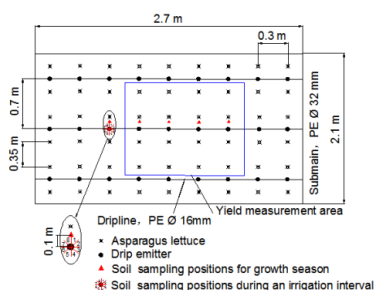


Fig. 1. Layout of driplines and sampling positions.

Soil samples were collected during the growth season (26 August, 7 October, 18 October, and 1 November) in 36 plots at four depths: 0 to 10 cm, 10 to 20 cm, 20 to 30 cm, and 30 to 40 cm. The positions of soil sampling were about 10 cm apart from an emitter. Moreover, additional *E. coli* time decay soil samples (27 September) for the surface irrigation treatment were picked in a sequential time (Fig.1). During the experiments, all samples were transported to laboratory in sterilized plastic containers (B01062WA, Nasco WHIRL-PAK, US) under low temperature condition about 4 °C. Soil samples were analysed for the population of *E. coli*. For each soil sample, 1 g of soil was transferred into 10 mL sterile phosphate-buffered solution (P1020, Solarbio, Beijing), shaken for 10 min and filtrated with a membrane (B5768-50MG, JINTENG, Tianjin). Then the membranes were moved into a plate with *E. coli*/coliform selective agar (BD, US). Plates were incubated at 37 °C for 24 h and the population of *E. coli* was enumerated.

Prior to harvest (29 October), three asparagus lettuce plants were picked from each treatment randomly and the samples were divided into stems and leaves for bacteriological analysis. For each sample, 5 g of leaves or stems were added into 50 mL sterile phosphate-buffered solution before shaken and filtrated. Then the population of E. coli was measured using the procedures mentioned above.

3. Results and discussion

3.1 Evaporation and irrigation

Fig. 2 shows the daily average temperature, evaporation and the irrigation applied during the growing season of asparagus lettuce. In general, the temperature decreased with time, and the evaporation also showed a decreasing trend. In total, six irrigations were applied during the entire growth season.

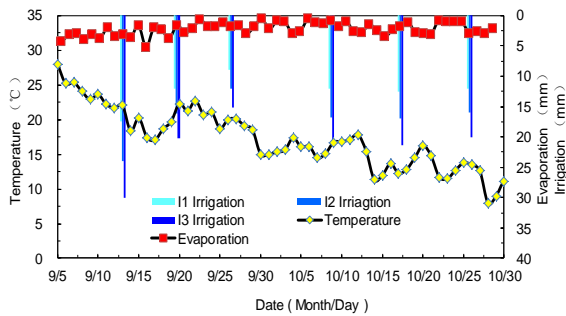


Fig.2. Evaporation, irrigation and daily average temperature.

3.2 E. coli in effluent and soil during asparagus lettuce growing season

During the growth season of asparagus lettuce, E.coli were detected on 13 September (700 CFU/100 mL), 27 September (100 CFU/100 mL) and 18 October (900 CFU/100 mL). These E. coli concentrations of the treated sewage effluent used in the experiment were larger than the value suggested by EPA guidelines (less than 25 CFU/100 mL, EPA, 2012). E. coli was not detected in groundwater samples.

Table 1. E. coli distribution in soil and asparagus lettuce samples. Table 1 presents the E. coli distribution in soil and asparagus lettuce samples.

Source	Sampling date	Depth (cm)	No. of E. coli positive samples	Total no. of samples	Maximum of E. coli (cfu/g)	Treatment of the maximum E. coli
Soil	26-Aug	0-10	1	36	1	I3D0
		10-20	0	36	0	
		20-30	0	36	0	
		30-40	0	36	0	
	7-Oct	0-10	1	36	1	I2D0C
		10-20	0	36	0	
		20-30	0	36	0	
		30-40	0	36	0	
	18-Oct	0-10	9	36	35	I3D0
		10-20	8	36	34	
		20-30	2	36	10	
		30-40	1	36	1	
1-Nov	0-10	3	36	1	I2D0	
	10-20	0	36	0		
	20-30	0	36	0		
	30-40	1	36	1		
Asparagus lettuce	1-Nov	Leaves	6	108	1	I1D0
		Stems	0	108	0	

coli in the surface soil layer but E. coli tended to distribute randomly in soil while sewage effluent was applied by subsurface drip irrigation (D1 and D2 treatments).

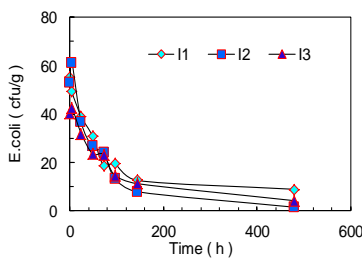


Fig. 3. E. coli decay in the 10 cm surface soil after an irrigation ceased.

Fig. 3 shows E. coli decay in the surface 10 cm soil for the surface drip irrigation (D0) treatments after the irrigation ceased for an event on 27 September. The concentration of E. coli in effluent was 1100 CFU/100 mL. E. coli decreased from 50-60 CFU/g to less than 15 CFU/g within an irrigation interval about a week, and decreased to left less than 9 CFU/g after 20 days following the irrigation. A similar decay trend of E. coli was found for the three irrigation levels tested. Kouznetsov et al. (2004) worked on microbial transport in soil caused by surface and subsurface drip irrigation with treated wastewater, and pointed out that a substantial die-off was observed during the second and third days of experiment with surface irrigation. Field research conducted by Fonseca et al. (2011) reported that the survival of bacteria in soil persisted longer in furrow irrigated areas, ranging from 17 days in winter months to 5 days during the warmer summer periods.

For asparagus lettuce on harvest, no E. coli was detected in stems, and only 6 of 108 leaves contained E. coli with a concentration less than 1 CFU/g. Similar result was reported by Forslund et al. (2012). They found that only two out of 84 tomato fruit samples contained E. coli when subsurface drip irrigation was used. They also pointed that the importance of the external environments, typically wildlife, as sources of bacterial contamination.

3.3 Yield

Table 2. *Asparagus lettuce yield and variance analysis.* The yields for all treatments ranged from 42 to 51 t/ha (Table 2). A

Treatments	Single plant weight (g)	Yield (t/ha)
I1D0	492 a	43 a
I1D1	530 a	46 a
I1D2	496 a	50 a
I2D0	504 a	48 a
I2D1	539 a	51 a
I2D2	538 a	51 a
I3D0	538 a	47 a
I3D1	455 a	50 a
I3D2	479 a	47 a
I2D0C	436 a	42 a
I2D1C	496 a	47 a
I2D2C	506 a	48 a
Two-way analysis of variance		
Irrigation level	I	NS (P=0.97)
Lateral depth	D	NS (P=0.54)
Irrigation level	I	NS (P=0.99)
Water quality	C	NS (P=0.67)

general but not statistically significant increasing tendency of yield with lateral depth for a given irrigation level and water quality was observed. For example, the yield averaged over the three irrigation levels for sewage application for the SDI treatments (49 t/ha) was about 7% higher than the surface drip irrigation treatments (46 t/ha). A possible reason was that subsurface drip irrigation systems delivered water and chemicals to the root zone of plants and improved water and fertilizer use efficiency. For a given lateral depth, a slight increase in yield was found when irrigation level increased from I1 to I2, but an approximately similar yield was observed for the I2 and I3 irrigation levels. This suggested that the pan coefficient of 0.8 be a suitable value for drip irrigated asparagus lettuce in greenhouse. For a given irrigation level, the average yield for the treatments irrigated with sewage effluent was 9% greater than that for the treatments irrigated with groundwater although the difference did not reach a statistical

significance level. More nutrients in sewage effluent than in groundwater might be a possible explanation.

4. Conclusion

E. coli had no significant increase in soil after irrigated with secondary sewage effluent. Subsurface drip irrigation appeared to be efficient in preventing *E. coli* contamination on soil surface. *E. coli* increased in the surface 10 cm soil layer after applying sewage effluent through surface drip irrigation, and the count was decreased to a low level within an irrigation interval. For all treatments, no *E. coli* was detected on the surface of stems, and only a few leaf samples had *E. coli* been detected, which showed that there was not an elevated risk of *E. coli* contamination of asparagus lettuce irrigated by either surface or subsurface drip irrigation with sewage effluent.

The subsurface drip irrigation treatments at a pan coefficient of 0.8 produced a higher yield. For a given irrigation level, drip irrigation applying sewage effluent produced a higher yield than groundwater irrigation. The result obtained from this research suggests that subsurface irrigation with secondary sewage effluent is effective in reducing *E. coli* contamination in soil and within plants and improving asparagus lettuce production.

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