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# Persistence of *Escherichia coli* O157:H7 in Major Leafy Green Producing Soils

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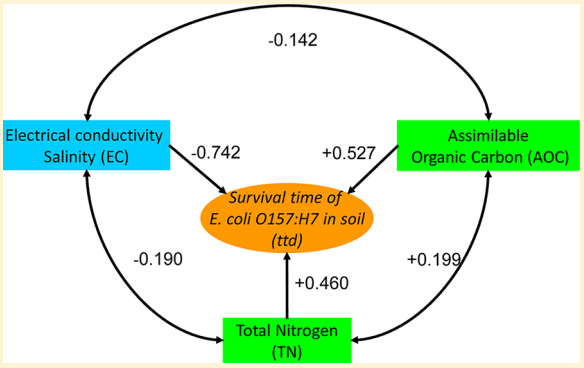
Supporting Information

**ABSTRACT:** Persistence of *Escherichia coli* O157:H7 in 32 (16 organically managed and 16 conventionally managed) soils from California (CA) and Arizona (AZ) was investigated. Results showed that the longest survival (*ttd*, time needed to reach detection limit, 100 CFU $g^{-1}$ dry soil) of *E. coli* O157:H7 was observed in the soils from Salinas Valley, CA and in organically managed soils from AZ. Detrended correspondence analysis revealed that the survival profiles in organically managed soils in Yuma, AZ were different from the ones in conventionally managed soils from the same site. Principal component analysis and stepwise regression analysis showed that *E. coli* O157:H7 survival in soils was negatively correlated with salinity (EC) ($P < 0.001$), while positively correlated with assimilable organic carbon (AOC) and total nitrogen (TN) ($P < 0.01$). Pearson correlation analysis revealed that a greater *ttd* was associated with a larger $\delta$ (time needed for first decimal reduction in *E. coli* population). EC was negatively correlated and TN was positively correlated ($P < 0.05$) with $\delta$, suggesting that EC and TN likely have a direct impact on *ttd*. On the other hand, AOC showed a close correlation with $p$ (the shape parameter) that was not directly related to *ttd*, indicating that AOC might have an indirect effect in the overall survival of *E. coli* O157:H7 in soils. Our data showed that AOC and EC significantly affected the survival of *E. coli* O157:H7 in leafy green producing soils and the development of good agricultural practices (manure/composting/irrigation water source management) in the preharvest environment must be followed to minimize foodborne bacterial contamination on fresh produce.




## INTRODUCTION

Shiga toxin-producing *Escherichia coli* O157:H7 is a food-borne pathogen that can cause watery diarrhea and hemorrhagic colitis.[1] Although most healthy adults can recover completely within a week without any medications, young children and the elderly could develop life-threatening hemolytic uremic syndrome.[2] Undercooked beef is believed to be the major cause of *E. coli* O157:H7 infection. However, accumulating evidence has shown that fresh produce serves as an important vehicle for the transmission of this pathogen,[3] and there is a growing burden of foodborne outbreaks due to contaminated fresh produce.[4,5] A recent multistate outbreak of *E. coli* O157:H7 caused by the consumption of contaminated fresh spinach resulted in 203 cases of illness including 97 hospitalizations and 3 deaths.[6] During 1995−2006, 22 outbreaks of *E. coli* O157:H7 infection associated with lettuce or spinach were reported in the United States, and 9 of these were traced to, or near, the Salinas Valley region of California, which is the major leafy green producing area in the U.S.[7]

Soil can also become contaminated through runoff from stored manure or manure applied directly to fields.[8−10] *E. coli* may persist and grow on fresh produce and, under favorable conditions, can be internalized into plant tissue, which potentially might enhance its survival.[11−13] Soil may act as a reservoir for *E. coli* and therefore, understanding of the survival behavior and sources of *E. coli* in soils will help in identifying management strategies to minimize soil contamination.

Depending on the soil properties and environmental factors, the survival of *E. coli* O157:H7 varies from one week to six months, and even longer in some extreme cases.[14−18] Numerous studies have been done to investigate the fate of *E. coli* O157:H7 in different agricultural systems with a major focus on the survival of *E. coli* O157:H7 in soils and manure-amended soils.[19−21] One of the most-recent review articles on survival of *E. coli* O157:H7 showed that temperature, soil structure, and microbial communities were the most important factors affecting survival.[9] Previous studies have revealed the negative impact of salinity and positive effect of organic matter on the survival of *E. coli*.[22,23] However, how soil salinity and more readily available organic carbon fraction in organic matter,

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e.g. assimilable organic carbon (AOC), affect the survival of the pathogens in soils are yet to be fully examined.

In this study, the survival of Shiga toxin-producing *E. coli* O157:H7 strain EDL933 in 32 soils from three major leafy green producing areas of California (CA) and Arizona (AZ) was investigated. The majority of *E. coli* O157:H7 outbreaks have been associated with Salinas Valley in CA where most of the summer (May−August) produce are grown.[7] While in Yuma Valley, AZ and Imperial Valley, CA the major produce are grown during winter (November−January). It is critical to identify the environmental factors that control the survival of *E. coli* O157:H7 in those areas. The major objective of current study is to investigate the role of salinity and assimilable organic carbon in soil water extract on the survival of *E. coli* O157:H7 EDL933.

## MATERIALS AND METHODS

**Soil Collection and Characterization.** A total of 32 soil samples were collected from three major fresh produce growing areas during fall of 2010: 8 from Salinas Valley, CA, 12 from Imperial Valley, CA, and 12 from Yuma Valley, AZ. Equal numbers of organically and conventionally managed soil samples were collected in each farm with close proximity and with similar soil types. The sampling Global Positioning System (GPS) coordinates, mean annual temperature (MAT), and mean annual precipitation (MAP), as well as other environmental parameters of sampling sites can be found elsewhere.[24] The major fresh produce growing on these locations during sampling were lettuce and spinach. Each sample (0−15 cm) was a composite of three individual soil cores taken at 5-m intervals, and triplicate samples were taken. Bagged samples were taken to the laboratory under ice. Vegetation, roots, and stones were removed, and the soil was sieved (<2 mm). Subsamples were air-dried for physical and chemical analyses according to standard methods including sand, clay, and silt content, and water holding capacity (WHC, %).[25] Total organic carbon (OC, %) and total nitrogen (TN, %) were determined using Flash 2000 NC Analyzers (Thermo Scientific, MA). Soil microbial biomass carbon (MBC, mg $kg^{-1}$) was extracted by the chloroform-fumigation-extraction method.[26] Water soluble organic carbon (WSOC, mg $kg^{-1}$) was measured by a total organic carbon analyzer (TOC-500, Shimadzu Corp., Kyoto, Japan) according to the method described previously.[27] Salinity or electrical conductivity (EC, dS $m^{-1}$) of each soil was obtained by determining the conductivity of soil water extract (30-min extraction in horizontal shaker with water to soil ratio of 1:1, vol:wt) using a conductivity meter (Okaton, IL). Concentrations (mM) of $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$ in soil water extracts were determined using an Optima-3300 DV ICP-OES spectrometer (Perkin-Elmer, MA) that was calibrated with certified standards prior to sample analysis.

Assimilable organic carbon (AOC) in soil water extracts was determined using the method developed recently.[24] Briefly, soil water extract was made by mixing 2 vol of Milli-Q water into the 1 vol of freeze-dried soil. The soil water extracts were inoculated with starved luminous cells of *Vibrio harveyi* BB721 (ATCC 700106) at late exponential phase. The AOC concentrations in soil water extracts were then quantified by relating the bioluminescence intensity of sample to that of the standard sample supplemented with known AOC (glucose) concentrations.

**Bacterial Strains.** The *E. coli* O157:H7 strain used was a derivative strain of *E. coli* O157:H7 EDL933 (ATCC 43895), which was originally isolated from raw hamburger meat implicated in a 1982 hemorrhagic colitis outbreak and has been well described and experimented in the literature.[1] To facilitate the enumeration of *E. coli* O157:H7 EDL933 on selective media, the EDL933 wild type was tagged with naidixic acid and rifampicin resistance, and its growth in LB (Luria−Bertani) broth and survival in soils with distinct textures were found to be identical to that of the nontagged wild-type strain.[16]

**Survival of E. coli O157:H7 EDL933 in Soils.** Inoculum preparation and inoculation followed the procedure reported previously.[16,19] Early stationary phase cells were used, and the cells were inoculated into the soil to a final concentration of $5 \times 10^6$ colony forming units (CFU) per gram of dry soil, which is the commonly used concentration in the literature.[19,24] All experiments were conducted under room temperature (22 ± 1 °C). Triplicate plastic bags containing the soils inoculated with *E. coli* O157:H7 EDL933 were prepared. Moisture content of the soil sample was maintained constantly (50% of WHC) during the course of experiment by adding more water to make up for evaporation. Soils without EDL933 were used as control. At days 0, 3, 6, 10, 14, 20, 27, 34, 40, and 48, the inoculated soils were sampled. Cells were extracted, plated on the selective agar plates, and finally, the CFU was counted according to the methods described previously.[16,19]

**Effect of Salinity (EC) on Survival of E. coli O157:H7 EDL933.** The effect of salinity on EDL933 in soils was tested by adding saline water of composition similar to Colorado River water,[28] which is the source water used to irrigate the soils in Yuma Valley, AZ and Imperial Valley, CA. The solution was added into 3 representative soils from each of the three regions as mentioned above. Inoculation of the *E. coli* O157:H7, sampling, and plate count procedures were the same as described above.

**Survival Data Modeling.** Survival of *E. coli* O157:H7 was analyzed by fitting the experimental data to the Weibull survival model using GInaFiT version 1.5 developed by Dr. Annemie Geeraerd.[29,30] The Weibull survival model was constructed based on the hypothesis that the deactivation kinetics of the *E. coli* O157:H7 EDL933 population follows a Weibull distribution.[19] The size of the surviving population can be calculated using equation 1,

$$\log(N_t) = \log(N_0) - (t/\delta)^p \quad (1)$$

where $N$ is number of survivors, $N_0$ is inoculum size, $t$ is time (days) post inoculation, $\delta$ is scale parameter representing the time (days) needed for first decimal reduction, and $p$ is nonunit shape parameter. When $p > 1$, a convex curve is observed, when $p < 1$, a concave curve is observed, and when $p = 1$, a linear curve is observed. Time needed to reach detection limit, in days (*ttd*) can also be calculated when using GInaFiT to fit the experimental survival data. The detection limit is 100 CFU per gram soil dry weight, $gdw^{-1}$.

**Statistical Analysis.** Principal component analysis (PCA) of soil physical variables, including EC (dS $m^{-1}$), WHC (%), silt content (%), clay content (%), bulk density (g $cm^{-3}$), and soil chemical variables, including TN (%), OC (%), WSOC, MBC (mg $kg^{-1}$), and *ttd* (day), were conducted using SYSTAT 12 (Systat Software, Chicago, IL). All environmental variables, except EC and pH, were $\log_{10}$ transformed. Detrended correspondence analysis (DCA) was performed using PC-ORD v5.0 (MjM Software, Gleneden Beach, OR), and survival parameters (*ttd*, $\delta$, and $p$), were used in DCA analysis. Stepwise

multiple regression analysis was performed using an Excel add-in tool (http://smgpublish.bu.edu/pekoz/addin/). In stepwise multiple regression analysis, the same soil properties as used in PCA were included. Path analysis was performed using SPSS 15 (SPSS Inc., Chicago, IL). Pearson correlation analysis of $\delta$, $p$, and *ttd* with soil properties (EC, AOC, and TN) was performed using SYSTAT 12 (Systat Software).

## RESULTS

**Sampling Sites Description and Soil Characterization.** A brief description of sampling sites and soil prosperities has been reported,[24] and EC, TN, AOC data are listed in Supporting Information Table S1. Yuma and Imperial Valley shared similar weather conditions, including MAT (23.9 vs 23.8 °C) and MAP (76.5 vs 74.2 mm), due to the closeness of the two locations in comparison to Salinas Valley. Soil pH was between 6.7 and 8.0. The salinity of conventionally managed soils from Yuma was significantly higher ($P < 0.01$) than that of organically managed soils. Also, soil salinity from Salinas Valley was significantly lower ($P < 0.01$) than those from Yuma and Imperial Valley.

**Survival Behavior of E. coli O157:H7 in Soils.** *E. coli* O157:H7 can survive for about 30 days before reaching the detection limit in soils from Salinas (31 vs 28.1 days in organically and conventionally managed soils, respectively) and about 20 days in soils from Imperial Valley (19.3 vs 18.4 days in organically and conventionally managed soils, respectively). No differences in survival (*ttd*) were observed in organically and conventionally managed soils from Imperial Valley and Salinas Valley (Figure 1A). In contrast, *E. coli* O157:H7 survived significantly longer ($P < 0.05$) in organically managed soils (25.7 days) from Yuma (AZ) than in conventionally managed soil (15.5 days) from the same location. Longest survival of *E. coli* O157:H7 (>1 month) was observed in soils collected from Salinas Valley, CA (Figure 1A). Longer survival coincided with higher $\delta$ value calculated from modeling of the survival data (Figure 1B). The shape parameter, $p$, was not different in the survival profiles of *E. coli* O157 in all soils (Figure 1C), with all the $p$ values larger than 1, except that the $p$ values calculated from survival data in AZ conventionally managed soils were smaller than 1. On the other hand, detrended correspondence analysis (Figure 2) was performed to visualize survival profiles of *E. coli* O157:H7 EDL933 in the soils. DCA results revealed that overall no difference in survival profiles of *E. coli* O157:H7 EDL933 in conventionally and organically managed soils, except the soils from Yuma, AZ, where the survival profiles in organically managed soils were well separated from those in conventionally managed soils collected from the same site. Moreover, the survival profiles of *E. coli* O157:H7 EDL933 in soils from Salinas Valley were well separated from the survival profiles of Imperial Valley and Yuma Valley.

**Principal Component Analysis of Survival Data and Soil Properties.** Many environmental factors had influence on the survival of *E. coli* O157:H7 in the soils studied. Principal component analysis was used to determine the effects of soil physical, chemical, and biological parameters on the survival of *E. coli* O157:H7. Principal component analysis results (Figure 3) showed that the first two PCs accounted for 69.0% of the total variance with PC1 accounting for 40.8% (Figure 3). According to PC1, TN, AOC, OC, MBC, and WHC exhibited a positive score, indicating that these factors may have positive effects on the pathogen survival, whereas silt, clay, pH, and EC had negative scores, indicating these factors may negatively affect the survival of *E. coli* O157:H7 EDL933 in soils.

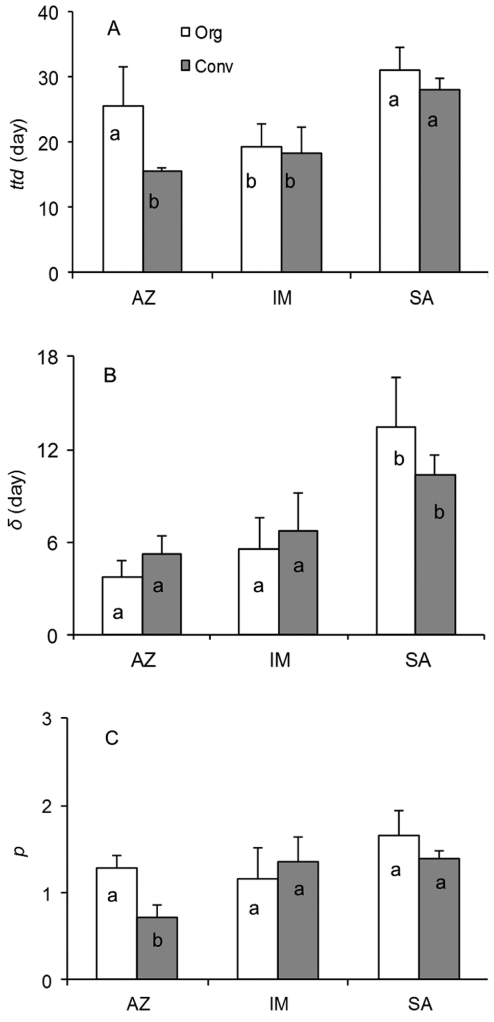


**Figure 1.** Survival of *E. coli* O157:H7 EDL933 in both organic (Org) and conventional (Conv) soils collected from Yuma, Arizona (AZ), Imperial Valley, CA (IM), and Salinas, CA (SA). Survival parameters resulted from the modeling of the raw survival data using Weibull deactivation model, including time needed to reach detection limit (100 CFU $g^{-1}$) (*ttd*), first decimal reduction time ($\delta$), and shape parameter ($p$), are shown in A, B, and C, respectively. Groups with the same letters are not significantly different from each other at the 0.05 level.

**Step-Wise Regression Analysis and Path Analysis of Survival Data and Soil Properties.** To quantify the results of PCA analysis, stepwise multiple regression analysis (Table 1) was conducted and the results showed that EC, TN, and AOC were the most important factors impacting the survival of *E. coli* O157:H7 in all soils tested, with EC showing the most negative effect ($P < 0.001$) on survival and TN and AOC showing positive effects ($P < 0.01$). For organic soils, TN and AOC showed the most positive influence ($P < 0.05$) on the survival of *E. coli* O157:H7, while in conventional soils, EC was the only factor negatively ($P < 0.001$) correlated with the survival of *E. coli* O157:H7. EC and AOC were the two factors significantly ($P < 0.05$) correlated with the survival of *E. coli* O157:H7 in clay soil, while in loamy soils, EC, pH, and TN were factors that significantly ($P < 0.05$) affected the survival of *E. coli* O157:H7. Furthermore, path analysis (Figure 4) evaluated the direct and

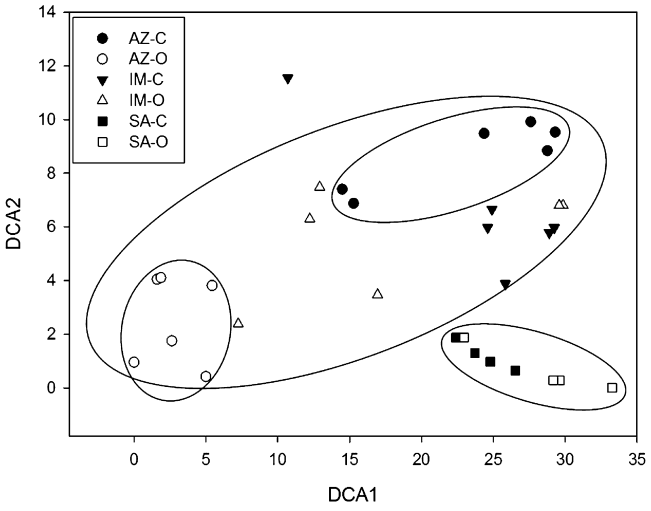


**Figure 2.** Detrended correspondence analysis (DCA) of the survival parameters; *ttd* (time to reach detection limit), $\delta$ (time needed for first decimal reduction in *E. coli* O157:H7 population), and $p$ (shape parameter) in soils collected from Yuma, Arizona (AZ), Imperial Valley, CA (IM), and Salinas, CA (SA). Both conventionally (C) and organically (O) managed soils were included. Closed and open symbols represent conventionally managed and organically managed soils, respectively.

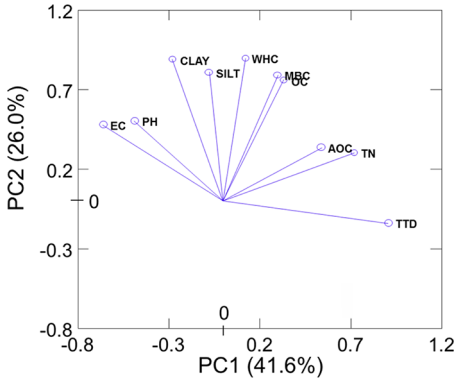


**Figure 3.** Factorial biplot by the principal components 1 and 2 (PC1, 41.6%; PC2 26.0%) resulting from the principle component analysis (PCA) performed on soil properties and survival (*ttd*) of *E. coli* O157:EDL933 in soils. Salinity (EC, dS $m^{-1}$), water holding capacity (WHC, %), silt content (%), clay content (%), total nitrogen (TN, %); OC, organic carbon (%); AOC, assimilable organic carbon in soil water extract (mg $kg^{-1}$); MBC, microbial biomass carbon (mg $kg^{-1}$).

indirect effect of the three most important environmental variables (EC, AOC, and TN) on *ttd*. All three parameters had strong direct impact ($P < 0.01$) on the survival of *E. coli* O157:H7 EDL933 in soils, and their indirect effect on the overall survival in soils was also noticeable although statistically not significant ($P > 0.05$).

**Effect of Salinity and Individual Cations on Survival of E. coli O157:H7 in Soils.** Linear regression analysis revealed that the survival (*ttd*) of *E. coli* O157:H7 in soils decreased significantly ($P < 0.05$) with increasing EC values in soil water extracts (Table 2). On average, survival decreased 1.7, 2.8, and 3.5 days when salinity was increased by one unit (dS $m^{-1}$) in the soil water extract of soils from Salinas, Yuma, and Imperial Valley, respectively (Figure 5). Further linear regression analysis (Table 2) revealed that *ttd* decreased significantly ($P < 0.05$) with increasing concentrations of individual cations such as $Na^+$, $Ca^{2+}$, and $Mg^{2+}$ in soil water extracts from all soils tested. In organic soils, only $Na^+$ and $Ca^{2+}$ were closely correlated to *ttd* ($P < 0.05$), while in conventional soils, all cations were correlated with *ttd* ($P < 0.001$). Sodium ion concentration was the most important factor affecting survival negatively in comparison to $Ca^{2+}$, $Mg^{2+}$, and $K^+$ based on regression coefficients. It was also shown that EC and the major cations correlated more negatively on survival of *E. coli* O157:H7 in conventional soil than in organic soils as indicated by $R^2$ and $P$ values (Table 2).

**Pearson Correlation Analysis of the Survival Parameters ($\delta$, p, and ttd) with Selected Soil Variables.** Pearson correlation (Table 3) of survival parameters ($\delta$, $p$, and *ttd*) with the selected soil variables (EC, AOC, and TN) showed that $\delta$ was positively correlated with *ttd* ($P < 0.05$), while $p$ had no direct correlation with *ttd*. EC was negatively correlated ($P < 0.05$) with both $\delta$ and $p$. It showed that AOC was negatively correlated with $p$ ($P < 0.05$) in soils, except in organically managed soils. Overall, TN was positively correlated with $\delta$ ($P < 0.05$), except in conventionally managed soils.

## DISCUSSION

In the current study, survival data were collected while the population size was above the detection limit of the method. The following data analysis, including Weibull modeling, DCA, PCA, regression analysis, and Pearson correlation were based on the survival data at or above the detection limit, and the data presented in the current study may only reflect the behavior of the *E. coli* O157:H7 at or above the detection limit. Our subsequent enrichment confirmed the presence of viable cells, i.e. the more resistant population; these data are semi-quantitative and therefore cannot be used in the model construction. Also, the behavior of the more resistant population might be different from the more sensitive one.

The survival data suggested that *E. coli* O157:H7 survived better in soils from Salinas Valley, CA, than Imperial Valley, CA and Yuma, AZ. The longer survival of the pathogen in Salinas Valley soils could be attributed to many factors that may include low levels of EC as one of the factors. As shown in Figure 3, EC was the most significant environmental factor negatively affecting survival. Electrical conductivity may be one of the factors contributing to the high survival rate in Salinas Valley soil and the high frequency of outbreaks caused by contaminated fresh produce traced back to Salinas Valley,[7] but not in other produce growing areas of the western United States (i.e. Imperial Valley, CA and Yuma, AZ). Other factors, such as heavier precipitation and runoff potential, as well as relatively low MAP in the Salinas Valley, compared to Imperial Valley and Yuma, may lead to changes in salinity and organic matter loading in leafy green growing farms, and ultimately might result in a relatively more frequent *E. coli* O157:H7 outbreaks associated with fresh produce from this area.

In the current study, multiple regression and path analyses generated similar trends in the relationships between *E. coli* O157 survival and soil properties. Our results showed that EC was the important abiotic factor influencing the survival of *E. coli* O157 in soils studied. EC of soils in southern CA is relatively higher, due to the high evaporation and low precipitation[31] compared to the values observed in soils from Salinas Valley. To the best of our knowledge, the influence of salinity (EC) on the survival of *E. coli* O157:H7 on leafy green production soils has not been studied before. The mechanisms of adverse effect of EC to the *E. coli* O157:H7 are not clear.

**Table 1. Stepwise Multi-Regression Analysis of Soil Properties and Survival Time (*ttd*, Time Needed to Reach Detection Limit, 100 Colony Forming Units Per Gram of Soil Dry Weight) of *E. coli* O157:H7 EDL933 in Soils[a]**

| soil group | regression equation | $R^2$ | *F* value | *T* value of partial regression coefficient | |
|---|---|---|---|---|---|
| Overall ($n$ = 32) | *ttd* = 23.27 (±1.76) − 5.35 (±0.74) × EC + 34.56 (±11.80) × TN + 1.07 (±0.25) × AOC | 0.79 | 35.47*** | EC | − 7.21*** |
| | | | | TN | + 2.92** |
| | | | | AOC | + 4.27*** |
| Org ($n$ = 16) | *ttd* = 13.78 (±12.33) + 55.27 (±18.94) × TN + 1.39 (±0.38) × AOC | 0.67 | 13.02*** | TN | + 2.92* |
| | | | | AOC | + 1.39** |
| Conv ($n$ = 16) | *ttd* = 28.40 (±1.09) − 6.01 (±0.0.65) × EC | 0.89 | 32.83*** | EC | − 5.18*** |
| clay ($n$ = 13) | *ttd* = 24.42 (±3.56) − 4.72 (±1.44) × EC + 1.21 (±0.41) × AOC | 0.76 | 16.00** | EC | − 3.28** |
| | | | | AOC | + 2.93* |
| loam ($n$ = 19) | *ttd* = 50.52 (±11.63) − 4.03 (±1.10) × EC − 3.69 (±1.57) × pH + 52.52 (±9.69) × TN | 0.87 | 34.96*** | EC | − 3.66*** |
| | | | | pH | − 2.32* |
| | | | | TN | + 5.42*** |

[a]Org, organic soil; Conv, conventional soil; *ttd* (d), time needed to reach detection limit (100 CFU $g^{-1}$ soil); EC (dS $m^{-1}$), electrical conductivity or salinity; TN (%), total nitrogen in soil; AOC (mg $kg^{-1}$), assimilable organic carbon in soil water extract; clay (%), clay content. Symbols *, **, and *** denote significance at the 0.05, 0.01, and 0.001 test levels, respectively. For *T* values, "+" indicates a positive correlation and "−" indicates a negative correlation.

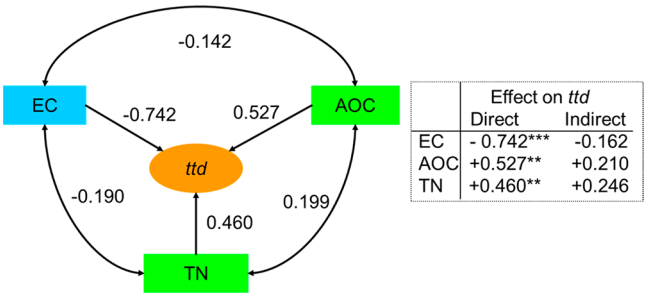


**Figure 4.** Path analysis of the effects of electrical conductivity (EC, dS $m^{-1}$), assimilable organic carbon (AOC, mg $kg^{-1}$) in soil water extract, and total nitrogen (TN, %) on the survival (*ttd* in day, time needed to reach detection limit) of *E. coli* O157:H7 EDL933 in soils. Each number shown represents the correlation coefficient between two neighboring parameters. The table on the right shows the direct and indirect effect of EC, AOC and TN on *ttd*. Symbols ** and *** denote significance at the 0.01 and 0.001 levels, respectively.

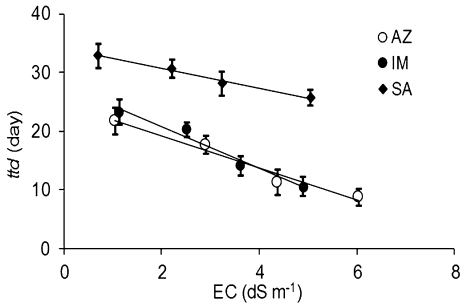


**Figure 5.** Survival of *E. coli* O157:H7 EDL933 in selected soils from three fresh produce-producing areas, Yuma, Arizona (AZ), Imperial Valley, CA (IM), and Salinas, CA (SA), supplemented with saline water (to increase EC, electrical conductivity salinity) simulated to Colorado River water composition. Error bars represent the ± 1 standard deviation of triplicate survival experiments.

However, the fact is that higher EC levels may produce shorter survival time of *E. coli* O157:H7 in soils (Figure 5). Previous research of salt stress on *E. coli* focused on commensal *E. coli* strains.[22] A recent study showed that salinity may adversely influence the survival of *E. coli* by a general osmotic effect or by specific ion toxicity, which leads to a decrease of activity in some enzymes that are essential for cell metabolism, e.g. $K^+$ transport.[32] Previously, Anderson et al. (1979) showed that survival of a commensal *E. coli* strain was comparable in seawater and in NaCl solutions of equal salinity.[22] This might also hold true for the survival of *E. coli* O157:H7 in soils. Soil salinity might interfere with ion transport, inhibit enzyme activity, and suppress synthesis of crucial proteins in *E. coli* O157:H7, and finally, result in a reduced survival capacity in soils. Several genes were shown to have a significant effect, e.g. *otsA*, *relA*, *spoT*, *ompC* and *ompF*,[33−35] among which *rpoS* was the most dominant.[34] A *rpoS* mutant was shown to decrease survival in seawater by 3 logs over 8 days.[34] It may be helpful to investigate the whole genome expression in response to salt stress toward a better understanding of the survival mechanisms of *E. coli* O157:H7 at the molecular level.

It is well-known that AOC is the most readily bioavailable organic carbon fraction among the organic matter pool, and is

**Table 2. Linear Regression Analysis between Survival Time (*ttd*, Time Needed to Reach Detection Limit, 100 Colony Forming Units Per Gram of Soil Dry Weight) with Salinity (EC) and Major Cations of Both Organic Soils and Conventional Soils[a]**

| | overall ($n$ = 32) | | | organic soil ($n$ = 16) | | | conventional soil ($n$ = 16) | | |
|---|---|---|---|---|---|---|---|---|---|
| | $R$ | $R^2$ | $P$ | $R$ | $R^2$ | $P$ | $R$ | $R^2$ | $P$ |
| EC (mS $m^{-1}$) | − 0.73 | 0.530 | <0.001*** | − 0.59 | 0.343 | 0.017* | − 0.93 | 0.859 | <0.001*** |
| $Na^+$ (mM) | − 0.72 | 0.522 | <0.001*** | − 0.55 | 0.301 | 0.028* | − 0.97 | 0.945 | <0.001*** |
| $Ca^{2+}$ (mM) | − 0.69 | 0.470 | <0.001*** | − 0.61 | 0.368 | 0.013* | − 0.80 | 0.638 | <0.001*** |
| $Mg^{2+}$ (mM) | − 0.63 | 0.399 | <0.001*** | − 0.49 | 0.238 | 0.055 | − 0.81 | 0.654 | <0.001*** |
| $K^+$ (mM) | − 0.37 | 0.136 | 0.038** | − 0.40 | 0.158 | 0.133 | − 0.73 | 0.539 | <0.001*** |

[a]Symbols *, **, and *** denote significance at the 0.05, 0.01, and 0.001 test levels, respectively.

Table 3. Pearson Correlation Analysis of the Survival Parameters ($\delta$, $p$, and $ttd$) and Soil Properties (EC, AOC, and TN)[a]

| soil group | | $p$ | $ttd$ | EC | AOC | TN |
|---|---|---|---|---|---|---|
| overall ($n$ = 32) | $\delta$ | + 0.838*** | + 0.641*** | − 0.690*** | − 0.019 | + 0.547*** |
| | $p$ | | + 0.166 | − 0.417* | − 0.363** | + 0.324 |
| org ($n$ = 16) | $\delta$ | + 0.921*** | + 0.605* | − 0.641** | + 0.086 | + 0.825*** |
| | $p$ | | + 0.287 | − 0.541* | + 0.196 | + 0.658** |
| conv ($n$ = 16) | $\delta$ | + 0.721** | + 0.940*** | − 0.905*** | − 0.563* | + 0.092 |
| | $p$ | | + 0.439 | − 0.546* | − 0.686** | + 0.213 |

[a] $\delta$, the time needed for first decimal decrease in *E. coli* O157:H7EDL933 population inoculated into the soils; $p$, the shape parameter; $ttd$, the time needed to reach detection limit (100 CFU $g^{-1}$ soil); EC, electrical conductivity salinity; AOC, assimilable organic carbon in soil water extract (soil to water, 1:1). Symbols *, **, and *** denote significance at the 0.05, 0.01, and 0.001 test levels, respectively.

an important indicator of bacterial regrowth potential in drinking water distribution systems.[35] The survival of human pathogens in drinking water sources is of particular interest among scientists in public health microbiology and water quality.[36] A recent study showed that AOC is one of the major determinants controlling the growth of the selected pathogenic bacteria, including *Escherichia coli* O157, *Vibrio cholerae*, and *Pseudomonas aeruginosa* in water.[37] Extending the AOC concept to soil is useful as it may provide an indication of the potential growth and survival of pathogens, and allocthonous microorganisms that are introduced into the soil with amendments, or other sources. It has been proved that organic carbon is directly related to the overall survival of *E. coli* O157:H7,[19] however, how the survival of this pathogen in soil correlates with the AOC levels in soils is still not clear. This is largely due to the lack of methods to determine the AOC in soil. Recently, we have developed a nongrowth based protocol to assess AOC in soil water extract.[24] In the current study, for the first time we reported that the AOC levels in soil water extract were positively correlated ($P < 0.05$) with $ttd$, indicating that a higher level of AOC might be associated with longer survival time for *E. coli* O157:H7 in soils just as was observed in drinking water sources.[37,38] It should be noted that elevated AOC in soil water extracts might not only promote the growth of the introduced human pathogens, but also the indigenous microorganisms that might suppress the persistence of the introduced pathogens. The net effect of AOC on $ttd$ might largely depend on how efficiently the pathogen competes for AOC pool with other microorganisms within the niche.

It was not surprising to find that TN was significantly correlated with the survival of *E. coli* O157:H7 in soils tested. Previous studies have demonstrated that $NH_4$-N and $NO_3$-N can promote the survival of the pathogens in soil. Obviously, there are many different N species in soil, including the more bioavailable N, such as amino acids, $NO_3$, and $NH_3$, which might have a more direct effect in promoting the persistence of the introduced pathogens into the soils. In the current study, we showed that TN was positively correlation with $ttd$, so additional work might be needed to examine the N fractions in the soils, and how different N fractions might influence the survival of *E. coli* O157:H7 in soils. A previous report has shown that dissolved organic nitrogen (DON) and $NO_3$-N positively related to the $ttd$ of *E. coli* O157:H7 in soils.[19] It would be interesting to investigate the effect of more bioavailable nitrogen, both in organic form (e.g., amino acids) and inorganic form ($NH_4$-N, $NO_3$-N), on the persistence of the pathogen in agricultural soils.

We found that the $\delta$ was significantly correlated with $ttd$, suggesting that $\delta$ might be a better bioindicator in assessing the persistence of *E. coli* O157:H7 in agricultural soils. The use of $\delta$ as a bioindicator would significantly shorten the time needed to get $ttd$. The $\delta$ values could be easily approximated by counting the survivors on a daily basis until the first decimal reduction is reached within two weeks, rather than $ttd$, which takes one month to be obtained. In the current study, we found that elevated EC levels significantly decrease $\delta$ values and TN significantly increase the $\delta$ values. When all soils were considered, AOC had no correlation with $\delta$ values, but had a significant relationship with the shape parameter, $p$. This suggests that the AOC fraction in soil water extract might stimulate the growth of indigenous microorganisms in addition to *E. coli* O157:H7 inoculated into the soils. Those microorganisms in turn might have a negative effect on the survival of *E. coli* O157:H7 in soils, resulting in a decrease of $p$ values. It should be noted that the $p$ values might not be a good indicator to determine the survival of *E. coli* O157:H7 in soils, since there was no significant correlation between $ttd$ and $p$.

Our data showed that abiotic factors such as EC, AOC, and TN might be important factors controlling the survival of *E. coli* O157:H7 in typical agricultural soils. However, it should be noted that other factors, e.g. environmental robustness between *E. coli* O157:H7 strains and structures of indigenous microbial communities, should also be considered when evaluating the survival of *E. coli* O157:H7 in agricultural soils. The overall survival of *E. coli* O157:H7 might be the result of a combination of physical, chemical, and biological factors. Our findings suggest high AOC and TN correlate with high survival of this pathogen and in theory therefore, decreasing AOC and TN may lower survival rate, or increasing the salinity level by changing the salt loading to the soils might be helpful in reducing the risk of on-farm contamination. This is very complex, and many factors must be considered as part of management practices in eliminating sources of pathogens to the growing environment.

## ■ ASSOCIATED CONTENT

### Supporting Information

Table S1: Soil properties. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

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