Controlling Foodborne Infections in Lettuce: Testing and Cleaning Methods for Curbing the Spread of *E. coli* O157:H7

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Abstract

E. coli O157:H7 bacteria tends to contaminate leafy green vegetable farms regularly, therefore promoting a recurrence in foodborne disease outbreaks. In previous studies, *E. coli* in water has been a focus. However, the farming industry is just now understanding the danger of *E. coli* in soil. In spring 2018, lettuce farms in the Yuma region contracted the *E. coli* bacteria and caused 210 human cases nationwide. As a result of this study, a new mathematical framework is proposed to capture the dynamics of the spread of *E. coli* in lettuce due to contaminated soil and equipment. In particular, this framework explores the impact of soil treatment and equipment sanitation since they are essential to the growing process of lettuce.

1 Introduction

The name *Escherichia coli* encompasses a large group of bacteria. Most strains of this bacteria are harmless to humans, but strains such as the Shiga toxin-producing *E. coli* (STEC) O157:H7 are pathogenic to humans [8]. In this study, mention of *E. coli* equates to the STEC O157:H7 strain. *E. coli* spreads to humans through contaminated food, usually ground beef and produce [9]. The Centers for Disease Control and Prevention (CDC) estimate that each year 96,534 individuals are infected with the strain STEC O157:H7 [25] and that 46% of outbreaks in the United States occur because of contaminated produce [5]. Leafy greens are the most common produce that are contaminated with *E. coli* [7], which is supported by the *E. coli* outbreaks from 2011, 2012, 2013, 2017, and 2018 [6]. The 2018 *E. coli* outbreak in romaine lettuce was linked to farms in the Yuma region. The commercial lettuce farms in Yuma, Arizona are responsible for 90% of the nations leafy greens [3] during the winter. As a result, this outbreak had wide reaching consequences. As of June 28, 2018, there were 36 states affected by the Yuma lettuce outbreak as seen in Figure 1. In this study, we focus specifically on the dynamics of the spread of *E. coli* in lettuce.

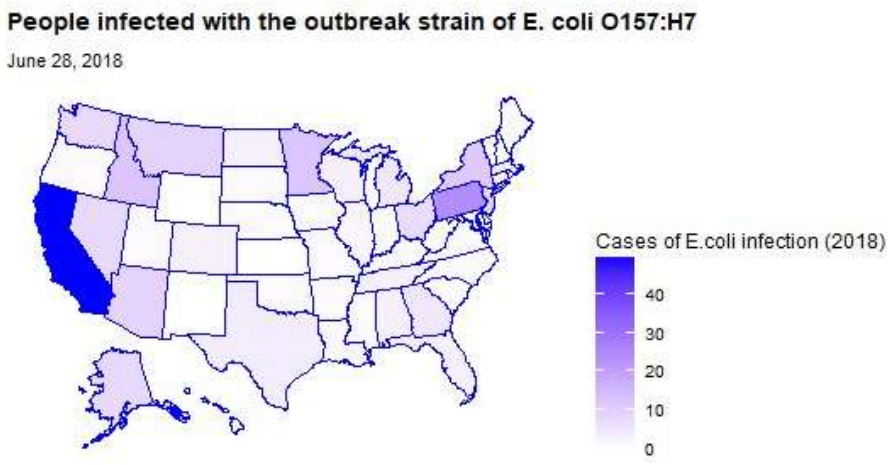

Figure 1: In this United States case count map of *E. coli*, a total of 210 human cases across 36 states were linked to romaine lettuce [18].

Although STEC O157:H7 is not the most common foodborne illness, it causes some of the most severe symptoms. When people consume *E. coli* contaminated food, they become infected and can experience severe symptoms such as bloody diarrhea, abdominal cramping, and vomiting [9]. Approximately, five to ten percent of *E. coli* cases lead to hemolytic-uremic syndrome (HUS) which can cause kidney failure and death [9]. Furthermore, these numbers do not encompass the full impact of the disease as cases are not reported when the illness is not linked to *E. coli* or the person does not go to the doctor. Only 20% of illnesses due to a foodborne outbreak are reported [8]. While the lettuce, itself, is unharmed by the presence of STEC O157:H7 [26], it can transmit *E. coli* to humans.

E. coli lives in the lower intestines of humans and animals as a part of the digestive system [23]. Cattle are the main source of contamination [10]. Infected cattle can contaminate their feces with *E. coli* from their intestines; this is called shedding. Cows can shed as many as 10^6 to 10^9 colony forming units (CFU) per gram of feces [16]. The waste from the infected cow is processed to use as manure for growing crops including lettuce. If this waste is improperly composted, it will still contain *E. coli* which will spread to the soil when manure is applied [17]. Consequently, this contaminated manure infects the lettuce that is being cultivated. Unknowingly, the *E. coli*-infected lettuce is grown, harvested, processed, and shipped out to stores for consumers to eat and become ill [17]. Farming equipment is an important mechanism of transmission [11]. Agricultural equipment enters the farming process at different times during the life cycle of the lettuce; they are used for the laying of manure, pre-planting ground preparation, planting, thinning, and harvesting [21]. Farm vehicles used in the growing season include the chisel plow, disk harrow, stanhay type planter, rotary spiker, and harvesting vehicle [21]. In addition, farm tools like long knives are used to cut heads of lettuce during harvesting. During these times, if equipment is used on contaminated soil or used to manipulate contaminated manure, it can transmit *E. coli* to uninfected soil and spread the infection.

There are several points of intervention throughout this process. Lettuce can be sanitized and disinfected within the normal process of preparing lettuce for consumers.

However, this will not be effective if the lettuce has internalized the bacteria. Internalization occurs when a lettuce seed is planted in contaminated soil and uptakes *E. coli* in its roots as it grows [26]. If this happens, then, it is nearly impossible to remove the bacteria from the lettuce. As a result, other points of intervention that prevent *E. coli* from coming into contact with soil at any point must be taken into account to limit internalization. One way to do so would be to prevent equipment from touching contaminated soil and manure.

Previous studies have shown that prevention during the preharvest process is crucial for reducing contamination. Past work by Franz, *et. al.* (2008) focused on the ecological factors that lead to the growth of STEC O157:H7 in lettuce. The probabilistic model concentrated on manure-amended soil through the production process. This study considered variables, such as herd density, manure storage time intervals, and manure quantity in order to estimate the probability of *E. coli* infected lettuce. The results show manure and soil management to be influential in preventing pathogenic *E. coli* in lettuce. Furthermore, the study states that there is a high correlation between the initial prevalence of contaminated manure and the probability of contaminated lettuce [17].

Our research focuses on testing and treatment methods to identify and treat contaminated soil and on sanitation methods of contaminated farm equipment through mathematical modeling. Some of the current procedures and standards of soil testing in a commercial farm setting include sending in soil samples to test in labs and at home soil testing kits [20]. If STEC O157:H7 is discovered in soil, then the soil is treated by no longer planting lettuce and letting the soil dry out from the sun via UV rays [20]. The standard for cleaning farm equipment consists of four stages. The first stage involves washing to loosen the soil on the surface. The second stage incorporates the use of detergent and scrubbing to break up the adhesion of the microorganisms. The next stage is rinsing that removes the loosened soil and detergent. The last stage is applying sanitizer to kill as much microorganisms as possible [27]. The frequency of sanitation is at the discretion of the farm, but the Food and Drug Administration (FDA) recommends that each farm develops their own sanitation standard operating procedures and schedules for sanitation [14].

As a result, we develop and analyze a mathematical model that describes the interaction of infected manure, contaminated soil, farm equipment, and lettuce. Our analysis uses sensitivity analysis of the basic reproduction number to determine the effect of treatment and sanitation on the yield of healthy lettuce.

2 Methods and Model

In our model, we first consider four state variables. The first two are clean equipment (E_c) and contaminated equipment (E_I). The other two state variables are healthy lettuce (L_S) and contaminated lettuce (L_I). The dynamics between the state variables are described in the following paragraphs.

We then assume a proportion of contaminated manure, ρ,is applied to the soil for fertilization. Consequently, we assume that *E. Coli* then colonizes the rest of the soil as a rate, r. In our model, the equation for the change in the proportion of infected soil, P, over time, t, is based off the Levins Model. The Levins model was developed to implement migration and extinction of a population in patches by utilizing a logistic growth equation [22]. We modified the Levins model to incorporate $M\rho$ into our model to reflect the proportion of infected manure that contaminates a proportion of soil at the start of cultivation. We assume that the infected manure infects the soil at the following rate,

$$rM\rho P(1-P).$$

Additionally, soil is treated at a rate $t_A P$ and the *E. coli* in the contaminated soil naturally dies out at rate dP. Manure and soil are essential in the growing process of lettuce.

The change in P over time t is shown below,

$$\frac{dP}{dt} = rM\rho P(1-P) - (d+t_A)P.$$

The germination of healthy lettuce plants occurs at a rate, αM. Germination occurs when a seed sprouts under favorable conditions such as appropriate water intake and temperature. We assume lettuce becomes contaminated in two ways. When the lettuce seed or plant comes into contact with infected soil, *E. coli* transmits to the lettuce at a rate of β_{L_S}. As a result, lettuce can internalize the *E. coli* from the soil and then the seed or plant becomes contaminated. In addition, healthy lettuce becomes infected when it comes in contact with infected equipment and the bacteria transmits at a rate $\hat{\beta}_{L_S}$. The per capita death rate of lettuce is μ_L which occurs at different stages of the growing season like during the thinning and harvesting process [21].

We consider farm equipment as our vector for *E. coli* spreading during the farming process. It is further assumed that new equipment such as tools and farm vehicles are acquired at a rate, Λ_E. Equipment moves from the clean, E_c, to contaminated, E_I, compartment when it comes in contact with *E. coli* contaminated manure and transmits *E. Coli* at a rate, β_M. Likewise, the term $\beta_{E_c} P \frac{E_c}{N_E}$, shows how clean equipment is contaminated as a result of its interaction with contaminated soil per unit time. The contact between equipment and soil occurs during the preplanting, thinning, fertilization, and harvesting stages. We consider all of the equipment and tools mentioned in Section 1 as the total population of equipment, N_E. The cleaning rate, γ_c, describes the rate at which a farmer randomly cleans infected equipment without knowing which ones are contaminated and moves equipment from the E_I to E_c compartment given that the farmer can only clean a certain amount of equipment per unit time, γ_c. However, if contaminated equipment cannot return to a clean state then it is discarded from the system at a rate, δ_E. Additionally, clean and contaminated equipment are considered to have gone through a per capita disposal rate, μ_E, when they are no longer usable. For example, dulled or broken knives that are discarded during harvest. Furthermore, homogeneous mixing of equipment and lettuce is assumed.

All of the dynamics are described in equations (1 – 4) and represented in Figure 2.

$$\frac{dP}{dt} = rM\rho P(1-P) - (d+t_A)P, \tag{1}$$

$$\frac{dE_c}{dt} = \Lambda_E + \gamma_c \frac{E_I}{N_E} - \beta_{E_c} P \frac{E_c}{N_E} - \beta_M M \rho E_c - \mu_E E_c, \tag{2}$$

$$\frac{dE_I}{dt} = \beta_{E_c} P \frac{E_c}{N_E} + \beta_M M \rho E_c - \gamma_c \frac{E_I}{N_E} - (\mu_E + \delta_E) E_I, \tag{3}$$

$$\frac{dL_I}{dt} = \beta_{L_S} P L_S + \hat{\beta}_{L_S} \frac{E_I}{N_E} L_S - \mu_L L_I, \tag{4}$$

$$\frac{dL_S}{dt} = \alpha M - \beta_{L_S} P L_S - \hat{\beta}_{L_S} \frac{E_I}{N_E} L_S - \mu_L L_S, \tag{5}$$

where $N_E = E_c + E_I$.

Because the total population of lettuce, N_L is assumed to approach $\frac{\alpha M}{\mu_L} = k$, the lettuce population is asymptotically constant.

From equation (1), we can see that P is between 0 and 1 because $\frac{dP}{dt}\big|_{P=0} = 0$, and $\frac{dP}{dt}\big|_{P=1} < 0$. From equation (2) and (3), we can see that $0 < E_c + E_I \leq \frac{\Lambda_E}{\mu_E} = k_E$

(carrying capacity of equipment). So the triangle region $\Delta = \{0 \leq E_I + E_c \leq k_E, E_I \geq 0, E_c \geq 0\}$ is positive invariant. Similarly, from equation (5 – 4), we know $0 \leq L_I \leq k$. Therefore, the domain of interest of our model is

$$\Omega = [0, 1] \times \Delta \times [0, k]$$

which is a positive invariant for the system (1 – 4). We will analyze the model within this domain.

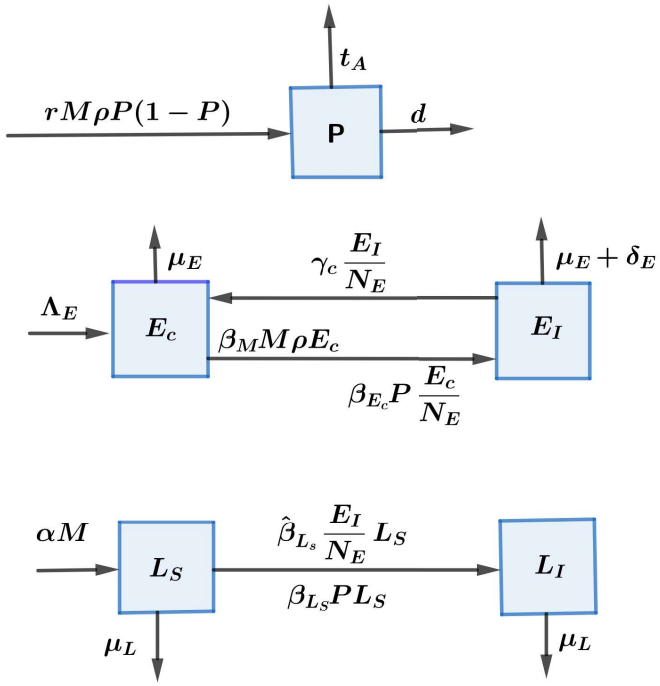

Figure 2: Flow diagram describing the interactions from equations (1 - 4). P is the proportion of infected soil, E_c clean equipment, E_I contaminated equipment, L_S healthy lettuce, and L_I infected lettuce.

3 Parameters

The first assumption we make for our parameters is that we define the size of a field to be 1 km^2. The Food Safety Modernization Act states that if a farm's water is known to be infected with *E. coli*, then the water is to be tested four times within a growing season [15]. We assume that this is the same for soil and calculated our treatment rate by dividing the number of times soil is tested by the number of days in a growing season, 152 days [21]. The parameter, δ_E, was calculated based on a strategy of some farmers for replacing their equipment. This particular strategy consists in replacing one or two pieces of equipment every year [19]; consequently, we divided the 2 pieces of equipment by the total number of days in a year to get, $\frac{2}{365} \approx 0.005$. Λ_E, the equipment acquired per day, is estimated to be some amount of equipment, less than 25. μ_E has to be

less than Λ_E, because when μ_E is computed it is multiplied by the total population of equipment. The cleaning rate of equipment, γ_c, was calculated based on the presumption that farmers clean 10 pieces of equipment weekly, biweekly, monthly, or never, which is shown by dividing 1 by the number of days between the cleaning, $\frac{10}{7}$, $\frac{10}{14}$, $\frac{10}{30}$, and 0 respectively. Therefore, the range of γ_c is between 0 and $\frac{10}{7}$.

Between 25,500 to 41,500 seedlings of lettuce are planted at the start of the lettuce growing season per acre [28], which we use to calculate the germinating rate of healthy lettuce, α, by first dividing by 0.004 to convert it to the number of lettuce per km^2, 6,375,000 to 10,375,000. Then, we divide 6,375,000 and 10,375,000 by the amount of manure times the number of days in our growing season, $\frac{6375000}{2300000*152} \approx 0.0182$ and $\frac{10375000}{2300000*152} \approx 0.03$. We can also use these numbers to compute the per capita death rate by multiplying the amount of manure by α and then dividing by the number of lettuce per km^2, $\frac{2300000*0.0182}{6375000} \approx 0.0066$ to $\frac{2300000*0.03}{10375000} \approx 0.0067$. All of the β values can be varied. The parameters, ρ and r are assumed to be proportions, 0 to 1. Parameter values M and d were found from other sources [17], [1] and the only computations involving these parameters are conversions to be consistent with the units used in this study.

Symbol	**Parameters**	**Estimations**	**Reference**
r	Growth rate of infected soil ($\frac{1}{kg \times day}$)	[0,1]	Estimate
M	Quantity of manure (kg)	$2,300,000$	[17]
ρ	Proportion of infected manure	$[0,1]$	Estimate
t_A	Treatment rate of soil as a result of testing ($\frac{1}{day}$)	Varies	Estimate
d	Per capita death rate of *E. coli* in soil ($\frac{1}{\text{day}}$)	0.3326	[1]
Λ_E	Rate equipment is acquired ($\frac{equipment}{day}$)	0.164	[19]
α	Germinating rate of healthy lettuce ($\frac{lettuce}{day \times mass}$)	$[0.0182, 0.03]$	[28]
β_{E_c}	Rate of transmission per equipment ($\frac{1}{equipment \times day}$)	Varies	Estimate
β_M	Transmission rate of manure ($\frac{1}{mass \times day}$)	Varies	Estimate
β_{L_S}	Contamination rate of healthy lettuce due to infected soil ($\frac{1}{day}$)	Varies	Estimate
$\hat{\beta}_{L_S}$	Contamination rate of lettuce due to infected equipment ($\frac{1}{day}$)	Varies	Estimate
γ_c	Cleaning rate of equipment ($\frac{equipment}{day}$)	Varies	Estimate
μ_E	Per capita disposal rate of equipment ($\frac{1}{day}$)	0.164	[19]
μ_L	Per capita death rate of lettuce ($\frac{1}{day}$)	0.0066	[28]
δ_E	Removal rate of infected equipment due to inability to clean ($\frac{1}{day}$)	Varies	Estimate

Table 1: Symbols, Definitions, and Parameter Estimates.

4 Analysis

In order to facilitate our mathematical analysis, we begin by re-scaling our model. Then, we find equilibria for the system and determine the conditions for their existence and stability as well as determining the important threshold, R_0^S expression. Finally, we examine the global dynamics for our system.

4.1 Re-scaled Model

Since $L_I + L_S = k$, we reduce the system (1 – 4) in terms of the following system of four equations

$$\frac{dP}{dt} = rM\rho P(1-P) - (d+t_A)P, \tag{6}$$
$$\frac{dE_c}{dt} = \Lambda_E + \gamma_c\frac{E_I}{N_E} - \beta_{E_c}P\frac{E_c}{N_E} - \beta_M M\rho E_c - \mu_E E_c, \tag{7}$$
$$\frac{dE_I}{dt} = \beta_{E_c}P\frac{E_c}{N_E} + \beta_M M\rho E_c - \gamma_c\frac{E_I}{N_E} - (\mu_E + \delta_E)E_I, \tag{8}$$
$$\frac{dL_I}{dt} = \beta_{L_S}P(k-L_I) + \hat{\beta}_{L_S}\frac{E_I}{N_E}(k-L_I) - \mu_L L_I, \tag{9}$$

where $N_E = E_c + E_I$ and $k = \frac{\alpha M}{\mu_L}$
The equations (6 – 9) are re-scaled using the following equivalences.

$$t = \frac{\tau}{\rho M r},\ P = \frac{\gamma_c}{\beta_{E_c}}x,\ E_c = \frac{\gamma_c}{\rho M r}y,\ E_I = \frac{\gamma_c}{\rho M r}z, \text{ and } L_I = kw.$$

The nondimensionalized system of (6 – 9) becomes

$$\frac{dx}{d\tau} = x(1-Ax) - G_0 x, \tag{10}$$
$$\frac{dy}{d\tau} = G_1 + \frac{z}{y+z} - \frac{xy}{y+z} - G_2 y, \tag{11}$$
$$\frac{dz}{d\tau} = \frac{xy}{y+z} + G_4 y - \frac{z}{y+z} - G_6 z, \tag{12}$$
$$\frac{dw}{d\tau} = G_7 x(1-w) + G_8\frac{z}{y+z}(1-w) - G_9 w, \tag{13}$$

where, $A = \frac{\gamma_c}{\beta_{E_c}}$, $G_0 = \frac{d+t_A}{\rho M r}$, $G_1 = \frac{\Lambda_E}{\gamma_c}$, $G_2 = \frac{\beta_M M\rho + \mu_E}{\rho M r}$, $G_4 = \frac{\beta_M}{r}$, $G_6 = \frac{\mu_E + \delta_E}{\rho M r}$, $G_7 = \frac{\beta_{L_S}\gamma_c}{\beta_{E_c}\rho M r}$, $G_8 = \frac{\hat{\beta}_{L_S}}{\rho M r}$, and $G_9 = \frac{\mu_L}{\rho M r}$.

The domain of interest, Ω, is changed to $\Omega' = [0, 1/A] \times \Delta' \times [0,1]$ where $\Delta' = \{0 \leq x + y \leq \frac{k_E M r \rho}{\gamma_c}\}$.

4.2 Contamination-Free Equilibrium of Soil

We will now do a full analysis of our re-scaled model (10–13). Firstly, the contamination-equilibrium of soil is ($x^* = 0$, y^*, z^*, w^*) where,

$$
\begin{aligned}
y^* &= \frac{-(1+2G_1)G_2 + G_4 + G_1(G_4+G_6)}{2G_2(G_4+G_6-G_2)} \\
&\quad + \frac{\sqrt{G_2^2 - 2G_2(G_4+G_1G_4-G_1G_6) + (G_4+G_1G_4+G_1G_6)^2}}{2G_2(G_4+G_6-G_2)} \\
z^* &= \frac{G_1 + (G_4-G_2)y^*}{G_6}, \\
w^* &= \frac{G_8 z^*}{G_9(y^*+z^*) + G_8 z^*}.
\end{aligned}
$$

Given that y^* exists, z^* exists if $y^* < \frac{\Lambda_E M r \rho}{\gamma_c \mu_E}$. Also, w^* exists if both y^* and z^* exist. To determine the stability of the contamination-free soil equilibrium, we find the Jacobian matrix

$$
\begin{pmatrix}
-G_0+1 & 0 & 0 & 0 \\
-\frac{y^*}{y^*+z^*} & -G_2 - \frac{z^*}{(y^*+z^*)^2} & -\frac{y^*}{(y^*+z^*)^2} & 0 \\
\frac{y^*}{y^*+z^*} & G_4 + \frac{z^*}{(y^*+z^*)^2} & -G_6 - \frac{y^*}{(y^*+z^*)^2} & 0 \\
G_7(1-w^*) & \frac{G_8(-1+w^*)z^*}{(y^*+z^*)^2} & -\frac{G_8(-1+w^*)z^*}{(y^*+z^*)^2} & -G_9 - \frac{G_8 z^*}{y^*+z^*}
\end{pmatrix}
$$

and, solve for the eigenvalues,

$$
\lambda_1 = 1 - G_0 \text{ and } \lambda_2 = -G_9 - \frac{G_8 z^*}{y^*+z^*}.
$$

After reducing the Jacobian to a 2×2 matrix:

$$
\begin{pmatrix}
-G_2 - \frac{z^*}{(y^*+z^*)^2} & -\frac{y^*}{(y^*+z^*)^2} \\
G_4 + \frac{z^*}{(y^*+z^*)^2} & -G_6 - \frac{y^*}{(y^*+z^*)^2}
\end{pmatrix}.
$$

In order to determine stability, we want to determine the conditions for when λ_1 and λ_2 are negative. In particular, $-G_0+1$ is negative when $G_0 > 1$ or $\frac{t_A+d}{Mr\rho} > 1$, and $-G_9 - \frac{G_8 z^*}{y^*+z^*}$ is always negative. To determine when the other two eigenvalues have negative real parts we check when the trace of the 2×2 matrix is negative and the determinant is positive. Obviously, the trace, $-G_2 - \frac{z^*}{(y^*+z^*)^2} - G_6 - \frac{y^*}{(y^*+z^*)^2}$ is always negative. The determinant

$$
\frac{G_2 y^* - G_4 y^* + G_6 z^* + G_2 G_6 (y^*+z^*)^2}{(y^*+z^*)^2},
$$

is positive because $G_4 < G_2$ since $\frac{\beta_M}{r} < \frac{\beta_M M \rho + \mu_E}{\rho M r}$. Therefore, the equilibrium is locally asymptotically stable when $G_0 > 1$. We collect the above analysis into the following theorem.

Theorem 1. *The contamination-free equilibrium of soil of system (10-13) is asymptotically stable when* $R_0^S = \frac{1}{G_0} = \frac{rM\rho}{t_A+d} < 1$.

Definition 1. R_0^S is the basic reproductive number for soil.

4.3 Endemic Equilibrium of Soil

The other equilibrium for equation (10) is $x_2^* = \frac{1}{A}(1 - G_0)$ which exists if $R_0^S > 1$. Let $(x_2^* = \frac{1}{A}(1 - G_0), y_2^*, z_2^*, w_2^*)$ be the endemic equilibrium of soil. Here, y^* is given by the following quadratic equation

$$ay^2 + by + c = 0 \tag{14}$$

where,

$$\begin{aligned}
a &= G_2(G_2 - G_4 - G_6) = -\frac{\delta_E(\beta_M M\rho + \mu_E)}{\rho M r}, \\
b &= G_6\left(G_1 - \frac{1}{A} + \frac{G_0}{A}\right) + G_4(G_1 + 1) - G_2 - 2G_1G_2 = \\
&\frac{\mu_E(\Lambda_E - \beta_{E_c} + \gamma_c - 2\Lambda_E\beta_M M\rho r) + \delta_E(-\beta_{E_c} + \Lambda_E) + \rho\beta_M\Lambda_E M}{\rho M r\gamma_c} \\
&+ (\mu_E + \delta_E)(d + t_A)(\beta_{E_c})(\rho M r)^2\gamma_c, \\
c &= G_1(G_1 + 1) = \frac{\Lambda_E}{\gamma_c}\left(\frac{\Lambda_E}{\gamma_c} + 1\right).
\end{aligned}$$

We know that $a < 0$ and $c > 0$, then the product of the two roots is less than zero. Therefore, equation (14) has one positive and one negative root and the positive root is the endemic solution. Then y_2^* is a positive root. We get

$$\begin{aligned}
y_2^* = &\frac{\sqrt{-4(-G_1 - G_1^2)(G_2^2 - G_2G_4 + G_2G_6) + (-G_1G_6 - \frac{G_0G_6 - G_6}{A} - G_4G_1 - G_4 + G_2 + 2G_1G_2)^2}}{2(G_2^2 - G_2G_4 + G_2G_6)} \\
&+ \frac{-G_1G_6 - \frac{G_0G_6 - G_6}{A} - G_4G_1 - G_4 + G_2 + 2G_1G_2}{2(G_2^2 - G_2G_4 + G_2G_6)}
\end{aligned}$$

and direct computation gives us

$$\begin{aligned}
z_2^* &= \frac{G_1 + (G_4 - G_2)y_2^*}{G_6}, \\
w_2^* &= \frac{\frac{(1-G_0)G_7y}{A} + \frac{(1-G_0)G_7z}{A} + G_8z}{\frac{(1-G_0)G_7y}{A} + G_9y + \frac{(1-G_0)G_7z}{A} + G_8z + G_9z}.
\end{aligned}$$

Since there exists a positive y_2^*, z_2^* is positive when the positive y_2^* is plugged in. In order for $z_2^* > 0$, the condition for existence $y_2^* < \frac{\Lambda_E M r\rho}{\gamma_c\mu_E}$ and $(x_2^*, y_2^*, z_2^*, w_2^*)$ exists when $R_0^S > 1$.

Similarly to the contamination-free equilibrium of soil stability, we can use a Jacobian to determine the stability of the endemic equilibrium corresponding to $x_2^* = \frac{1}{A}(1 - G_0)$

$$\begin{pmatrix} -1+G_0 & 0 & 0 & 0 \\ -\frac{y_2^*}{y_2^*+z_2^*} & \frac{(-1+G_0)z_2^*-A(z_2^*+G_2(y_2^*+z_2^*)^2)}{A(y_2^*+z_2^*)^2} & \frac{(1+A-G_0)y_2^*}{A(y_2^*+z_2^*)^2} & 0 \\ \frac{y_2^*}{y_2^*+z_2^*} & \frac{z_2^*-G_0z_2^*+A(z_2^*+G_4(y_2^*+z_2^*)^2)}{A(y_2^*+z_2^*)^2} & -G_6+\frac{(-1-A+G_0)y_2^*}{A(y_2^*+z_2^*)^2} & 0 \\ G_7(1-w_2^*) & -\frac{G_8(1-w_2^*)z_2^*}{(y_2^*+z_2^*)^2} & -\frac{G_8(-1+w_2^*)y_2^*}{y_2^*+z_2^*)^2} & \frac{(-1+G_0)G_7}{A}-G_9-\frac{G_8z_2^*}{y_2^*+z_2^*} \end{pmatrix}.$$

Two eigenvalues of this 4×4 matrix are

$$\lambda_1 = -1 + G_0 \qquad \text{and} \qquad \lambda_2 = \frac{(-1+G_0)G_7}{A} - G_9 - \frac{G_8 z_2^*}{y_2^* + z_2^*}.$$

The eigenvalues λ_1 and λ_2 are negative with the condition that $G_0 < 1$. Similar to the contamination-free equilibrium of soil, we can analyze a simplified 2×2 version of the Jacobian matrix

$$J = \begin{pmatrix} \frac{(-1+G_0)z_2^*-A(z_2^*+G_2(y_2^*+z_2^*)^2)}{A(y_2^*+z_2^*)^2} & \frac{(1+A-G_0)y_2^*}{A(y_2^*+z_2^*)^2} \\ \frac{z_2^*-G_0z_2^*+A(z_2^*+G_4(y_2^*+z_2^*)^2)}{A(y_2^*+z_2^*)^2} & -G_6+\frac{(-1-A+G_0)y_2^*}{A(y_2^*+z_2^*)^2} \end{pmatrix}.$$

Since the two outermost eigenvalues are negative, we only need to show that the trace of the 2×2 matrix is negative and the determinant is positive for the endemic equilibrium to be stable

$$Trace(J) = \frac{(-1+G_0)z_2^* - A(z_2^* + G_2(y_2^* + z_2^*)^2)}{A(y_2^* + z_2^*)^2} - G_6 + \frac{(-1-A+G_0)y_2^*}{A(y_2^* + z_2^*)^2} < 0$$

when $G_0 < 1$. The determinant is

$$Det(J) = \frac{y_2^*((1+A-G_0)(G_2-G_4) + AG_2G_6y_2^*) + G_6(1+A-G_0+2AG_2y_2^*)z_2^* + AG_2G_6z_2^*}{A(y_2^* + z_2^*)^2}$$

Since we have already set the condition that $G_0 < 1$ and $G_4 < G_2$, the determinant is positive. Based on the condition that $R_0^S > 1$ then $Trace(J) < 0$ and $Det(J) > 0$. Therefore, the endemic equilibrium is stable.

Theorem 2. *The endemic equilibrium of soil of system (10-13) is asymptotically stable when* $R_0^S = \frac{1}{G_0} = \frac{rM\rho}{t_A+d} > 1$.

4.4 Global Dynamics

The contamination-free equilibrium of soil, $x^* = 0$ and the endemic equilibrium of soil $x_2^* = \frac{1}{A}(1 - G_0)$ are globally stable when $R_0^S < 1$ and $R_0^S > 1$ respectively. By the limiting equation theorem [4], we can substitute x^* and x_2^* into equations (11 – 12). Equations (11 – 12) are a closed planar system. If we can rule out that our system has closed trajectories in our domain of interest, Ω', then our local equilibrium stability becomes globally stable. We can prove this by using the Dulac Criterion [2, 172].

Theorem 3. *Dulac's Criterion: If $D(y,z)$ in C^1 in a region $B \subseteq \mathbb{R}^2$ (simply connected) and $\frac{\partial}{\partial y}(DF) + \frac{\partial}{\partial z}(DG) \neq 0$ in B, then $y' = F$, $z' = G$ has no periodic orbits contained in B.*

Theorem 4. *There is no closed trajectory for system (11 – 12).*

Proof. We use the Dulac function to analyze whether equations (11) and (12) have a limit cycle. By making $D(y,z) = \frac{1}{y+z}$, then we get

$$\frac{\partial}{\partial y}(DF) + \frac{\partial}{\partial z}(DG) = -\frac{1 + G_1 + x + (G_4 + G_6)y + G_2 z}{(y+z)^2} < 0.$$

□

Hence, we do not have a limit cycle or closed trajectories and (y^*, z^*) is global. Because this is true, w's value is also global because equation (13) is a one dimensional system.

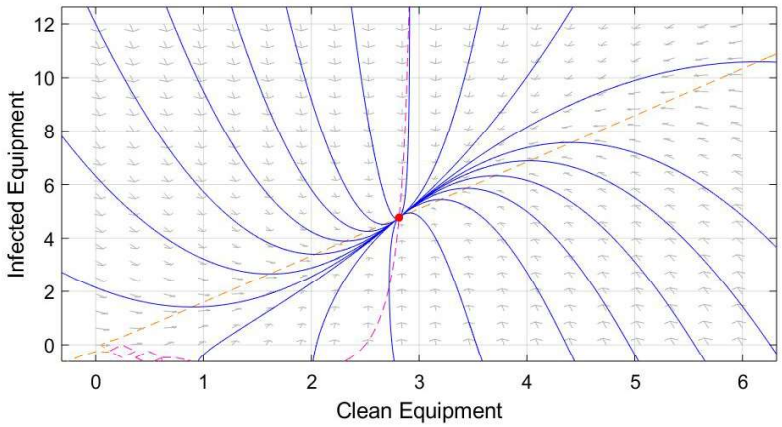

Figure 3: Global phase portrait of system (11 – 12) when $x^* = 0$

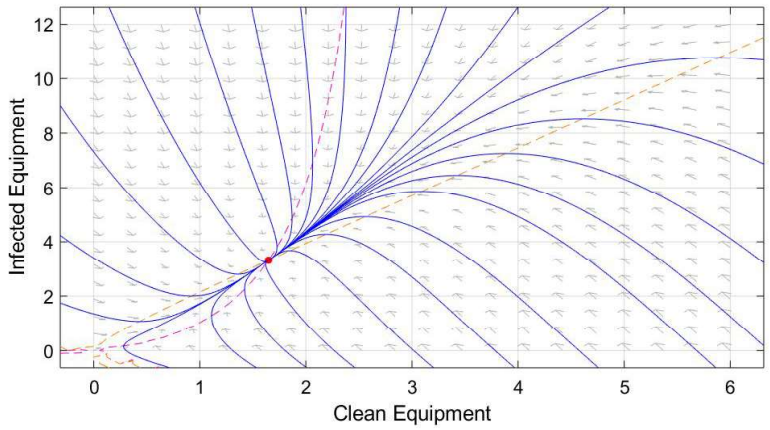

Figure 4: Global Phase Portrait of System (11 – 12) when $x^* = \frac{1}{A}(1 - G_0)$

Correspondingly, the phase portraits in Figure 3 and 4 show that the equilibria for equations (11 – 12) are globally stable for contamination-free equilibrium of soil and endemic equilibrium of soil respectively. This means that the trajectories will always head toward a positive equilibria for both contamination-free soil ($x^* = 0$) and contaminated soil equilibria $\left(x^* = \frac{1}{A}(1 - G_0)\right)$ regardless of the initial values.

5 Results

5.1 Impact of Control Parameters on R_0^S

We look at how the control parameters impact R_0^S. Figure 5 shows how R_0^S is influenced by t_A and ρ. The graph of R_0^S intersects with the flat plane where $R_0^S = 1$. The line that is generated by the intersection of planes is shown in Figure 6, which suggests that as the treatment rate of soil increases, the proportion of contaminated manure required to maintain $R_0^S = 1$ increases. We interpreted that as the proportion of infected manure increases, the need for treatment increases in order to get $R_0^S < 1$. The region above the line represent when the treatment is sufficient enough relative to the proportion of infected manure for R_0^S to be less than 1. The region below the line represents points for when the amount of treatment is not sufficient for the proportion of contamination in the manure. In this region, $R_0^S > 1$.

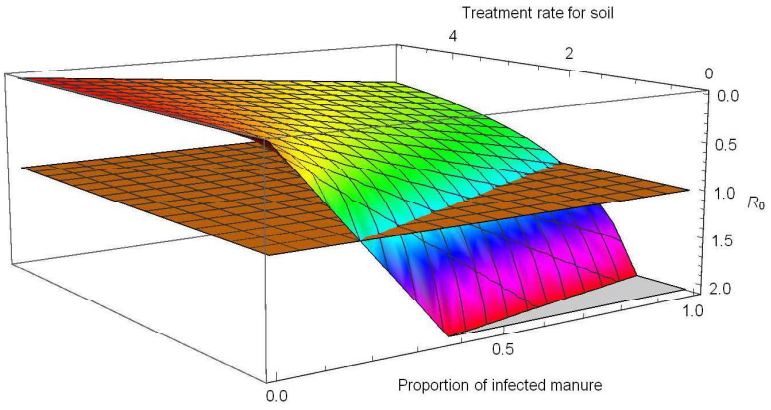

Figure 5: Graph of R_0^S as a function of t_A and ρ.

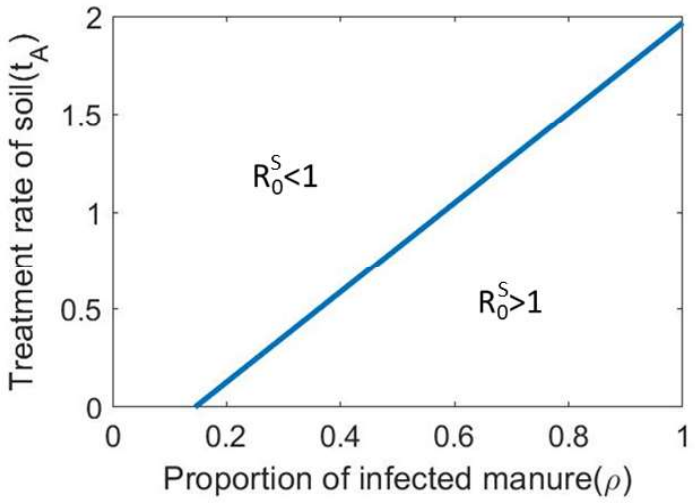

Figure 6: Bifurcation curve of R_0^S in (ρ, t_A) – plane.

5.2 Sensitivity Analysis

We carry out a sensitivity analysis on R_0^S with respect to M, r, ρ, t_A, and d. In Figure 7 the most significant parameters are M, ρ, and r, which increase R_0^S. However, an increase in t_A and d will decrease the R_0^S, which is favored to reach a contamination-free equilibrium of soil.

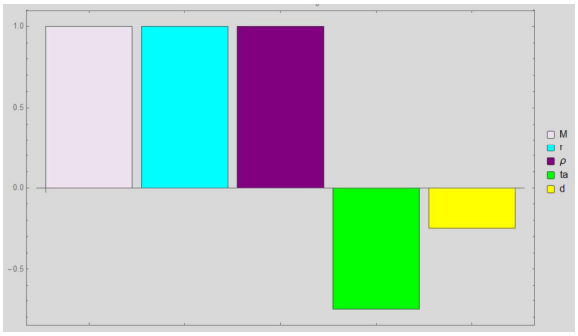

Figure 7: Sensitivity indices of R_0^S with respect to M, r, ρ, t_A, and d.

Figure 8 gives the sensitivity indices for the endemic equilibrium for P^*. As the values for r and M increase, P^* increases. When the values for t_A and d increase the value of P^* decreases. A smaller value for P^* means there is a smaller proportion of infected soil.

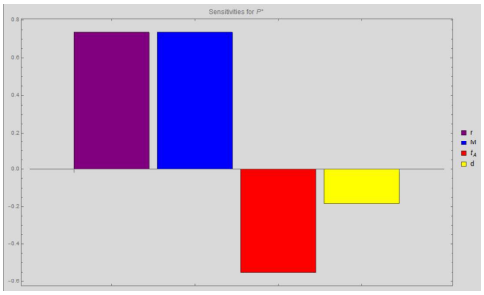

Figure 8: Sensitivity index of P^* with respect to r, M, t_A, and d.

Figure 9 describes the endemic equilibrium for E_c^* and E_I^*. The most significant parameter that increases equipment is δ_E, while r decreases equipment.

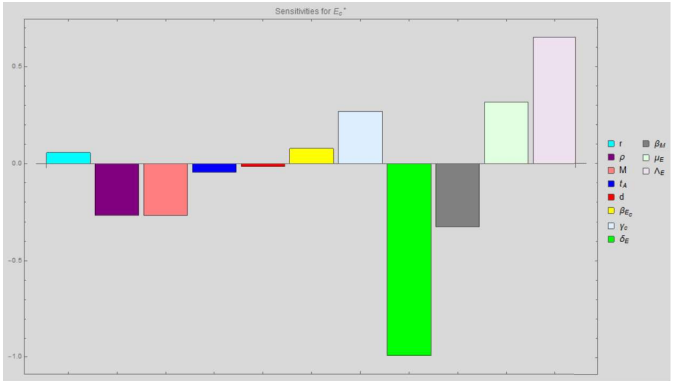

Figure 9: Sensitivity index of E_c^* with respect to r, ρ, t_A, d, β_{E_c}, γ_c, δ_E, β_M, μ_E, Λ_E

The sensitivity indices for contaminated lettuce, L_I^*, are very small when looking at the y–axis. The values for M and α are much larger than all the other parameters. Therefore, we separated M and α from the rest of them. We see that an increase in M, α has the greatest impact on L_I^* when compared to the other terms as can be seen in 10.

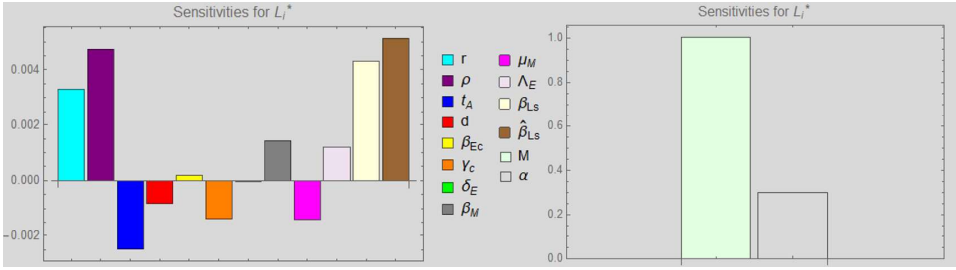

Figure 10: Sensitivity index of L_I^*. The values for parameters M and α are much larger than the rest so the two were extracted to their own plot (right), while the rest are shown on the left.

5.3 Simulations

We determine that increasing the cleaning rate of equipment will result in a decrease in the proportion of infected lettuce, as depicted in the nonlinear curve in Figure 11.

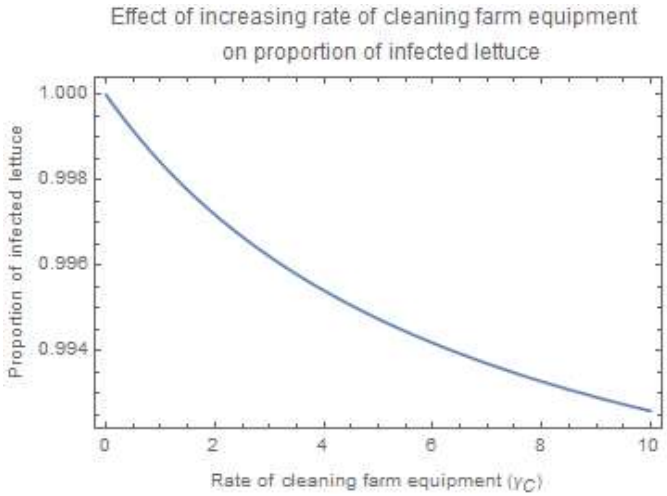

Figure 11: As the cleaning rate (γ_c) increases, the number of contaminated lettuce decreases.

Similarly, in Figure 12 we can show that the higher the rate of testing and treatment of soil for *E. coli*, the lower the proportion of infected equipment. Figure 13 shows this can lead to a higher population of healthy lettuce. Next, we run simulations of the proportion of infected soil, equipment, and lettuce over a time span of 20 days changing parameter values.

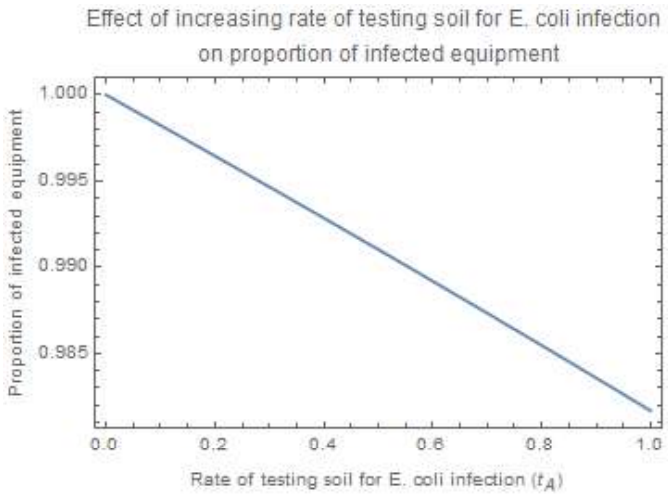

Figure 12: As treatment rate (t_A) increases, the number of contaminated equipment decreases.

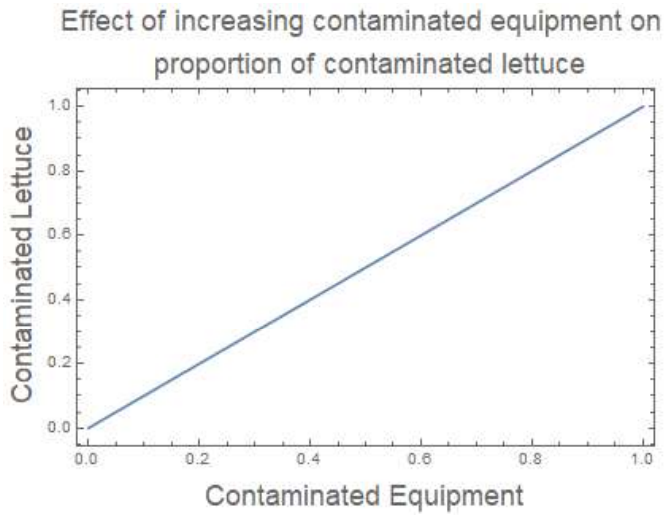

Figure 13: As contaminated equipment increases, contaminated lettuce increases.

In order to increase the amount of clean equipment, the removal rate of contaminated equipment due to inability to clean (δ_E) is increased. In Figures (14 – 16) the initial conditions for the proportion of infected soil, cleaned equipment, contaminated equipment, healthy lettuce and contaminated lettuce are $P_0 = 0.9$, $E_{c_0} = 9$, $E_{I_0} = 1$, $L_{S_0} = 1$, $L_{I_0} = 0$, respectively for all the simulations. In addition, the following parameters are $\rho = 9.91 \times 10^{-7}$, $r = 0.09$, $t_A = 0.9$, $\gamma_c = 10/7$, $\beta_{E_c} = 1$, $\beta_M = 0.5$, $\beta_E = 1$, $\mu_E = 1$, $\beta_{L_s} = 0.5$, $\mu_L = 0.9$, $\hat{\beta}_{L_S} = 1$, $\mu_E = 1$, $\Lambda_E = 10$, $\delta_E = 0.005$, and all other values (M, d, β_{L_S}) come from table 1. Hence $R_0^S < 1$ and as a result the proportion of infected soil eventually reaches zero as seen in Figure 14. In Figure 15, there are more clean equipment than contaminated equipment and Figure 16 shows that healthy lettuce is dominating.

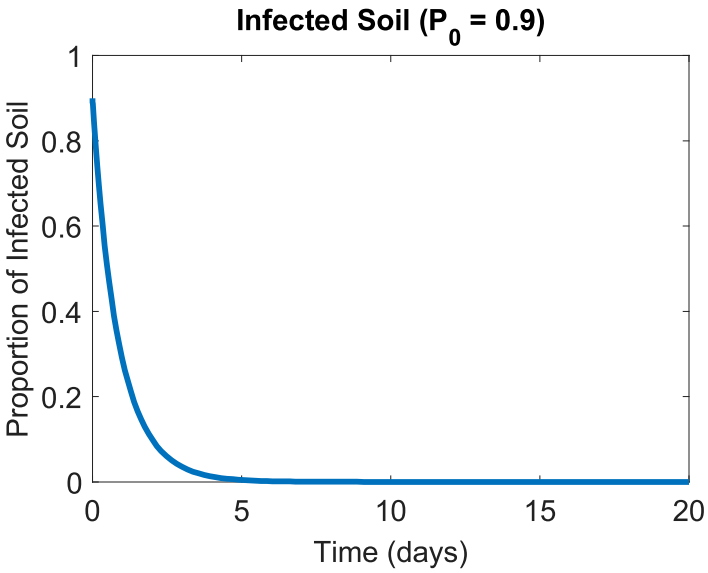

Figure 14: Infected soil over time.

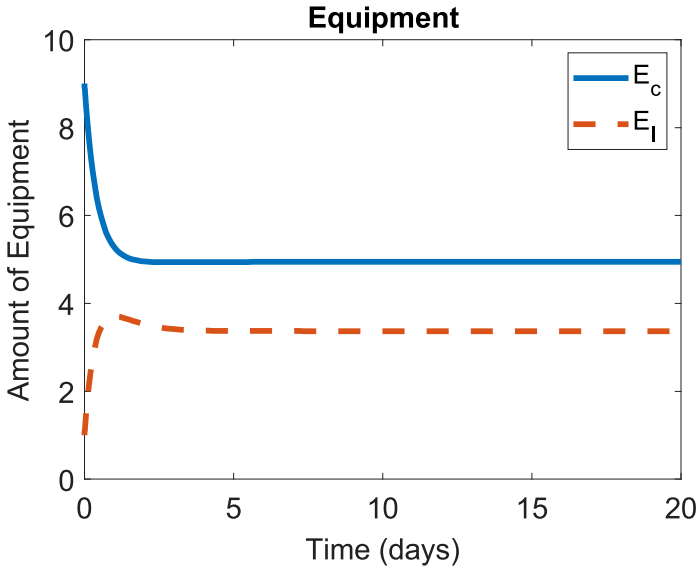

Figure 15: Equipment over time.

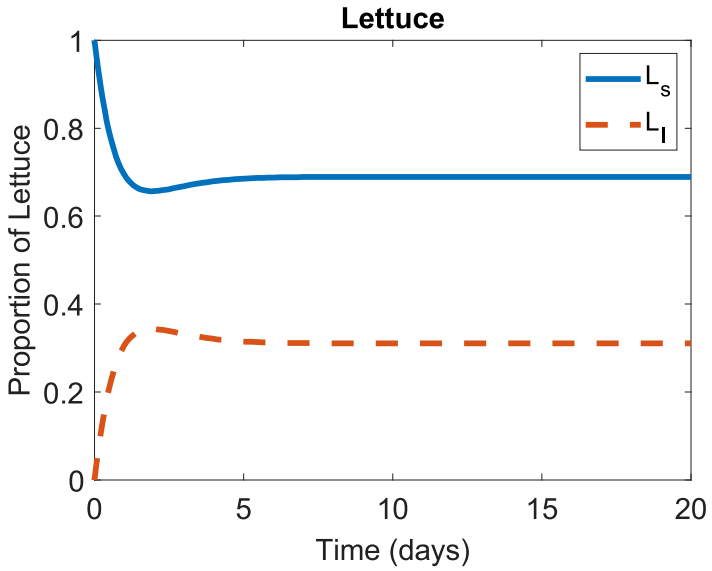

Figure 16: Lettuce over time.

6 Discussion

Similar studies investigated the probability of *E. coli* infection in lettuce, as well as prevention strategies, to reduce the chance of contaminated produce. Specifically, an article by Franz et al. (2008), focuses on modelling the likelihood that manure-amended soil from cattle infects lettuce. As a result, the "density of *E. coli* O157:H7 in manure-amended soil at the time of planting lettuce was most highly correlated to the storage time of the manure...and the initial concentration in manure..." [17]. This conclusion corresponds with our model in that once the manure becomes contaminated, then inevitably, the lettuce will become contaminated as well. Therefore, prevention efforts are suggested to take place early in the growing process. Examples would include proper composting and setting a minimum manure storage time to decrease the probability of infection. Another study concentrates on different models that help indicate the existence of *E. coli* O157:H7 in lettuce fields. When a scenario analysis was applied to a stochastic model, results showed that as time passed, the percentage of *E. coli* contaminated units decreased in the population [24]. In the same way, our simulation in Figure 14 shows that when $R_0^S < 1$, the proportion of infected soil decreases over time and eventually tends to 0.

Using our basic reproductive number for soil, $R_0^S = \frac{Mr\rho}{t_A+d}$, we can analyze the impact of testing and treatment of soil on the *E. coli* infection. Figures 5 and 6 indicate the t_A values for when the *E. coli* infection will die out in manure, meaning that more testing is needed to keep *E. coli* from becoming endemic. The positive correlation between ρ and t_A in Figure 6 demonstrates how much testing is required to reduce ρ in order for $R_0^S < 1$. In addition, Figure 7 illustrates that R_0^S is sensitive to t_A in a negative way such that when t_A is increased, the value of R_0^S becomes smaller. This is further evidence that testing and treatment of soil decreases *E. coli*; therefore, also impacting the yield of healthy

lettuce. Furthermore, in analyzing Figure 8, we know that the proportion of infected soil, P, is influenced by t_A, similar to the impact of t_A on R_0^S. Not only does t_A affect R_0^S and P, but also the proportion of clean equipment. As previously demonstrated, when t_A is increased, P decreases which reduces the proportion of contaminated equipment, as shown in Figure 12. The collective influence of testing and treatment of contaminated soil proves that t_A is an important parameter in controlling the spread of *E. coli* and preventing the infection from reaching lettuce.

The cleaning of contaminated equipment is another parameter that can aid in increasing the yield of healthy lettuce. Based on Figures 9 – 10, we know that E_c is positively impacted by γ_c and L_I is negatively effected by γ_c which is what we would expect to see. This indicates that by increasing the cleaning rate of equipment, we can control the spread of the *E. coli* infection from reaching the population of healthy lettuce. As the cleaning rate of farm equipment increases, there is also a decline in the proportion of contaminated lettuce; therefore, increasing γ_c helps to prevent *E. coli* from transmitting across the lettuce field and promotes the growth of healthy lettuce, as shown in Figure 11.

In addition to t_A and γ_c being control parameters in our system, ρ is also a parameter that has an overall effect. If we can reduce the value of ρ, then the proportion of infected soil, number of contaminated equipment, and number of contaminated lettuce are all reduced.

There are limitations in our study due to the lack of information; therefore, it was challenging to accurately determine parameters and fully represent the dynamics of *E. coli* in soil. Although we were unable to find exact transmission parameters, we used our best judgment to estimate values based on information from literature. Additionally, it was difficult to find current standards for testing and treatment of the *E. coli* infection in soil. However, this information may be available in the future because the FDA announced a projected start date for farms to incorporate regular water and soil testing in spring 2019 [12]. Finally, to more accurately capture the complexity of the transmission of *E. coli* to lettuce, other factors could be considered in our model such as the role of cattle and irrigation as well as soil nutrition. Despite these limitations, the development of our model has still given us insight into how *E. coli* interacts with different elements of a farm setting including manure, soil, and equipment. The lack of studies on this topic indicates that in the future there needs to be more research on the role of equipment in the transmission of *E. coli* on farms.

In future studies, we would like to incorporate irrigation water to see how this mechanism contributes to the spread of *E. coli* in lettuce. The irrigation system is of high interest because of the water contamination risk. It is common for canals and rivers to be near farms, so it serves as a water source in the planting season. Sprinklers are used routinely during preharvest to assist the growth of lettuce and could potentially include serious contaminants like the Shiga toxin-producing *E. coli*. In 1995, the foodborne outbreak in lettuce was linked to *E. coli* bacteria found in irrigation water in Montana [13]. Cattle feces, surface runoff, and groundwater can enter into nearby water sources and further infect water that is used for irrigation [7]. Additionally, we can include more terms such as the infection in soil because of contaminated equipment into equation (1) to aid in capturing the complexity of *E. coli*. Finally, we could conduct a cost analysis on several scenarios within an *E. coli* outbreak. One scenario is what could happen to a farm when it receives bad publicity from an outbreak and the monetary impact of that on the farm. Another scenario we could analyze would be to optimize costs of testing and treatment of contaminated soil and cleaning of contaminated equipment for farmers in order for them to prevent an *E. coli* infection on the farm.

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