2-1-2012

Effects of temperature and soil organic content on the growth and survival of E. coli in sandy soil

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Effects of Temperature and Soil Organic Content on the Growth and Survival of E. coli in Sandy Soil

BY

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A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science in Environmental Engineering
Under the Supervision of:

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January 2012

Dedication

I would like to dedicate this work to my family and my close friends in gratitude to all their patience, tolerance, support, and help to me. Without their efforts, I would not have made it this far and my success would not have been possible. I would also like to dedicate this work to my sister Diana, my best friend Yomna, because without their aid and unfailing love to me, I wouldn’t have been able to complete this research.

Acknowledgments

Without the support, help, and guidance of Professor Edward Smith, my supervisor, and my very patient and tolerant advisor, I would not have been able to complete or present this work. Dr. Smith, I would like to give you my eternal and unfailing gratitude for everything you have done for me and for your patience with me. Also, to Engr. Ahmed Saad, without whose help and support during my experimental work, thank you for everything. Without your help to me, I would not have been able to make it this far.

Abstract

With the continuous growth of populations and expansions in developing countries, the availability of sufficient water resources is approaching a critical state, especially in arid and semi-arid lands. In Egypt, although the Nile has been sufficient for many centuries now, its dependability for all life applications for the coming decades is in question. By far, agricultural practices consume the greatest portion of fresh water from the Nile. As a result, there is a growing effort dedicated to investigating the use of treated wastewater for irrigation instead of using virgin fresh water as a best-sustainable practice.

When it comes to the use of treated wastewater in agriculture, the contamination of highest concern is microbiological (bacteria such as *E-coli*, viruses, protozoa, and fungi). Not only does the direct application (*i.e.* without treatment) of wastewater before application pose great risks on the health of workers and the local community involved, but it also poses a high risk of contamination of the groundwater and the harvested crops. However, to what extent the wastewater should be treated before irrigation is the question that needs to be properly answered for the relevant site-specific conditions: while under-treatment renders the water unsafe, over-treatment can be costly and economically impractical. This study is a small part of a larger investigation that seeks to inform the development of guidelines for the sustainable use of treated wastewater in agriculture based on microbial contamination (using *E-coli* as an indicator) in a host environment representative of arid and semi-arid environments (sandy desert soil and desert outdoor conditions).

The extent and rate of growth of microbes as well as their decay rates is greatly affected by the host environment, which in such a case is the soil media properties (such as the amount of organic content in the soil) and the temperature and exposure to sunlight. To accomplish this, bacteria survival experiments were conducted in static soil column tests set up in the laboratory before exposure to outdoor conditions. The bacterial growth was studied for three different initial buffer concentrations repeated in the summer and the winter for soil with three different organic fractions (0.035%, 0.3%, and 0.5% respectively). Samples were then taken at different time frames throughout each experiment, which in most cases lasted for a week.

The study showed that in most cases, the total bacterial cells would reach their peak value within one day (24 hours). The extent of growth as well as the rate of growth and decay was considerably dependent upon the soil organic fraction and the temperature. At lower temperatures, the growth of the bacterial cells was observed to increase up to three orders of magnitude their initial value, and they were also observed to have more prolonged survival and slower inactivation rates. During the summer, on the other hand, the higher temperatures often promoted a more rapid die-off rate due to more intense solar radiation, decrease

in moisture, and faster decomposition rate of soil nutrients. The concentration profile within a column was often observed to vary more during summer than winter experiments.

A strong correlation was observed between bacterial growth and survival and the organic fraction of the soil. This was noticed in the change in the relative total cells of the bacteria in the soil column, where the highest peaks occurred at higher organic fractions. The increase in the organic content of the soil also tended to prolong the time of survival of the bacteria in soil even at high temperature.

As anticipated, the extent of *E. coli* growth in the test soil was directly proportional to the concentration of cells in the solution added to the soil columns. The results of this study should aid in the development of sustainable practices for the cultivation of the deserts using treated wastewater in order to minimize risks to human health and the environment in addition to providing data to calculate those risks. The results should also aid in determining more realistic guidelines for acceptable levels of pathogens in treated wastewater to be used in desert reclamation projects in arid regions like the Middle East because they account for site-specific variables unique to these environments.

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1 Introduction

The ongoing decline of many vital non-renewable natural resources such as fossil fuels and fresh water has led to the emergence and application of the concepts of sustainable use of resources and sustainable development. Of these concepts, three seem to be most important and applicable; reduction, reuse, and recycling. The first implies the necessity of water conservation through the reduction of any present overuse and exhaustion of the fresh water resources through planning and implementing long term conservation programs. Accordingly, establishing and implementing a well-planned conservation program can potentially decrease the consumption of water up to 10% – 20% within a period of 10 – 20 years, and with such conservation it would also be possible to meet future demands that seem difficult to achieve today (Maddaus, Gleason, & Darmody).

The other two concepts of reuse and recycle are in many cases used interchangeably, especially when water is the resource involved. Reuse essentially denotes the process of using the same product or resource without changing it into any other form, whereas recycle refers to the process of changing a certain product or resource through repairs or completely changing it into a whole different product. With water, the recycle can therefore be thought of as treatment in case of wastewater or desalination in case of sea water, whereas the reuse is using the wastewater, for instance, without changing or treating it. Identifying the necessity or the extent of treatment needed for wastewater in order to put these two concepts into actions depends on its type and its viability for use; while the source of the wastewater determines the extent of its toxicity. Depending on the types of contaminants and toxins in the wastewater, the kind(s) of treatment to be employed or the quality to be reached if indeed treatment is needed (physical, chemical, and/or biological) can be determined. For instance, if the target application is agriculture, progressive treatment processes might be needed in order to remove chemical constituents that are toxic to some plantations as well as the removal of some of the harmful microbiological pollutants. On the other hand, if the target application of the wastewater reuse is industrial, some chemical constituents might be prioritized such as the removal of constituents that are hazardous in their toxic or corrosive nature (Stephan & Weinberger, 1968).

The cultivated area in Egypt, which is considered an arid land where most of it is desert, is adjacent to the Nile River banks, its branches and canals, and the Nile Delta (FAO AQUASTAT, 2009). Accordingly, the green area is very small and only limited to the “narrow strip” along the Nile and along the coast of the Mediterranean, with width of only a few kilometers, and only about 3% of the whole country terrain (estimated in 2002 to be around 3.4 million ha that is dedicated to the cultivation of arable as well as permanent crops) (FAO AQUASTAT, 2009).

Expansion of the green area has been, since the early 1990s, considered one of the greatest challenges in Egypt. Studies were conducted with the goal of expanding the cultivated land from the Nile bank to the deserts through desert forestation development projects, in order to reduce the amounts of imported timber (whose costs was estimated to be about 3 billion Egyptian pounds per year) (Loutfy, 2010). This accordingly called for the use of another source of water than the fresh water of the Nile. **Table 1-1** shows some of the areas intended for forestation, and for which treated wastewater needs to be used for irrigation.

Table 1-1 - Wastewater-irrigated forest plantation pilot projects in Egypt (MWRI/USAID, 2000)

Site Names	Area (feddans)	Planted Trees	Soils	Irrigation Methods
Ismailia	500	Caprrisus and Pinus	Sandy Desert	Drip
Sadat	500	Caprrisus, Mulberry, and Pinus	Sandy Desert	Drip
Luxor (close to airport)	1000 (including a nursery for Mahogany Seedlings)	African Mahogany (Khaya)	Sandy Desert	Modified flood (a new area uses drip irrigation)
Qena	500	Eucalyptus and Mahogany	Sandy Desert	Modified flood
Edfu	500	African Mahogany	Sandy Desert	Modified flood
New Valley (El Kharga)	800	Eucalyptus, African Mahogany, and Terminalia	Sandy Desert	Modified flood
New Valley (Paris)	50	African Mahogany	Sandy Desert	Modified flood
South Sinai	200	Acacia and Eucalyptus	Sandy Desert	Drip
Abu Rawash	50	Experiment of Neem trees (controlling for insects)	Sandy Desert	Modified flood

1.1 Water Resources in Egypt

The major water resource in Egypt is of course the river Nile, which is used for almost all applications from agriculture and industry to drinking. Since 1959 under the Nile Waters Agreement, the established annual flow of the Nile in Egypt is 55.5 km^3/year (Abdel Wahaab & Omar).

The annual amount of the internal renewable surface water resources, however, is about 0.5 km^3, which makes the actual value of the total renewable surface water resources about 56 km^3 / year. Other water resources in Egypt include internal renewable groundwater that is estimated to be about 1.3 km^3/year, and which comes mainly from the soil filtration of irrigation water in the Nile valley and delta, but since the overlays between surface and groundwater resources are quite insignificant, the total value of the

renewable water resources in Egypt can be rounded up to a total of 57.3 km^3/year (FAO AQUASTAT, 2009).

The total annual amount of water withdrawn from the Nile for direct usage was estimated in 2000 to be around 68.3 km^3, of which an annual amount of about 59 km^3 is used only for agriculture (which is about 86% of the total), an annual amount of about 5.3 km^3 for municipal use (typically 8% of the total), around 4.0 km^3 per year that is for industrial usage (about 6% of the total), and an amount of 4.0 km^3 was used for navigation and hydropower (FAO AQUASTAT, 2009).

In the Upper Egypt region (in the south of the country), all the water from drainage flows back to the Nile as well as the irrigation canals, which has an annual amount of about 4 km^3, whereas the annual amount of drainage water flowing into the Nile from the Delta is estimated to be 14 km^3. The amount of municipal wastewater that is subject to treatment was estimated in 2001/02 to be around 3 km^3 per year (FAO AQUASTAT, 2009). The reuse of wastewater from drainage was estimated in 2001/02 to have an annual amount of 4.84 km^3. Of this amount, a total of 1.5 km^3 per year only is used for agriculture, whereas the remainder flows back into mainstream drains to be remixed again with drainage water. The wastewater that is treated and used for irrigation is mainly utilized in the landscaping of trees in urban areas as well as the trees planted along the roads, but the portion of treated wastewater being reused in irrigation is less than one-third (FAO AQUASTAT, 2009). The point is, there is a considerable opportunity to improve the water resources profile in Egypt by reusing a greater portion of the treated wastewater in agriculture.

1.2 Sustainable Water Management and Wastewater Reuse in Agriculture

The usage of the provisional fresh water resources from rivers, lakes, wells, or even rain is not divided evenly between the needs for different applications: drinking, domestic, industry, and irrigation. In Egypt, for instance, a very small percentage of the water from the Nile, which is the main source of fresh water used for most applications, is actually used for drinking because most of this water is used in agriculture (about 86%). In general, there is a growing need and an apparent shortage of freshwater that is especially critical in the arid and semiarid countries around the world. In the Middle East and North Africa (MENA), 16 out of 29 countries have been categorized as water-deficient, with only less than 500 m^3 per capita of the annual renewable fresh water resources (FAO, 1997a). Even though the MENA represents only 14% of the world area, and comprises nearly 10% of the world population, it receives only 3.5% of the total precipitation and about 2.2% of the annual internal renewable water resources. As in Egypt, so also across the MENA, where agriculture is the major water consumer constituting about 91% of the total

mobilized water, whereas other applications such as municipal and the industrial consumption represent only about 5% and 4% respectively (FAO, 1997a). As such, the reuse of treated wastewater in agriculture has moved to the center stage of policy discussion and activity in the MENA region in view of stressed freshwater sources, development goals, and the growing need for environmental protection (Bazza, 2003; Al Salem & Abouzaid, 2006).

With the continuous increase in population and the corresponding expansion in developed land in arid countries such as Egypt, the need for fresh water increases in accordance, and the problem of water shortage becomes more evident throughout the region. This makes the need to apply the concept of sustainable development more imperative, and heightens the search for alternative water resources for the major consumers of fresh water. The treatment and reuse of wastewater has been one of these alternatives being pursued by researchers, engineers, decision-makers, and planners for water resources in an endeavor to decrease the growing disproportion between the supply and demand of fresh water. Specifically, instead of using virgin freshwater in agriculture that consumes the most, wastewater can be used for irrigation after treatment to ensure safe and sustainable reuse. Different conditions apply based on the extent of contamination of the wastewater to be reused as well as the intended reuse application. If appropriate management is implemented for the reuse of reclaimed wastewater in agriculture, not only would it make a huge impact on the conservation of fresh water resources, but it may as well afford many additional benefits: economic and environmental. The vital nature of these issues is reflected in the large number of studies that describe their impact on wastewater reuse in agriculture and the associated risks. These will be elaborated in Chapter 2. For now, it is important to present the case for investigating biological contamination issues associated with irrigation with treated wastewater.

1.3 Reclaimed Wastewater Contamination and Health Risks

Reclaimed wastewater, in its raw form, contains a diverse amount of contaminants: microbial contaminants, heavy metals, as well as other toxic chemical constituents. This contamination, especially microbial contamination, poses a risk to public health and may cause several water-borne diseases; thus, necessitating effective and controlled treatment (Lund, 1978). According to a 2004 report from the Center for Environment and Development for Arab Region and Europe (CEDARE), untreated or poorly treated wastewater is being disposed in the Mediterranean posing a risk of its contamination, and therefore risking the health of community exposed to it (CEDARE, 2004). This is shown in **Table 1-2.**

The source of the wide range of microbiological contaminants existing in municipal wastewater could be both man and animals, and some of these microbes could survive in raw wastewater for

a very long time. The concentration of these microbiological contaminants present in the wastewater is influenced by many complex features. It is therefore hard to guarantee that the reclaimed water could be of one general pathogenic character or the other. To be able to provide such a characterization, one needs to know not only the sources of the wastewater contributing to its contamination but also to understand the relative ability of the various microbes existing in the reclaimed wastewater to survive outside its host when exposed to a range of different environmental circumstances.

Table 1-2 - Impacts of Disposed Wastewater on the Mediterranean in Some Arab Countries

Volume of wastewater/million cubic meters	Egypt	Syria	Jordan	Morocco	Tunisia
Treated and disposed in the Mediterranean	73				25
Untreated and disposed in the Mediterranean	12,000	210		40	50
Impacts on the Mediterranean (high-med.-low)	High	Low	Nil	Low	Low

The types of pathogens that can be found in raw municipal wastewater and cause water-borne diseases can be categorized in four general sets: bacteria, protozoa, helminthes, and viruses. Some of the most important bacterium causing diseases to man that can be found in wastewater typically include the *Vibrio Cholerae* that causes Cholerae-paracholerae, *Salmonella typhi* that causes typhoid fever, *E-coli* that causes enteritis, *Shigella flexneri* and others that cause para-dysentery, and *Mycobacterium tuberculi* that causes tuberculosis (Lund, 1978). Protozoan and helminthes organism that carry harmful diseases and can be found in wastewater include *Entamoeba histolytica* (protozoa) that causes Amoebic dysentery, *Giardia lamblia* that causes Giardiasis, *Schistosoma* that causes Schistosomiasis, and Nematodes such as *Ascaris* that causes Ascariasis (Lund, 1978).

Safe use of the wastewater in practices such as irrigation entails avoiding any public health risks involved such as these epidemics caused by organisms found in raw or inefficiently treated wastewater. Therefore, there is no question regarding the need to reduce the concentration of pathogenic contaminants before it can be used in agriculture. The issue is: what extent of treatment is needed and what limits of contamination will allow for safe reuse of wastewater in irrigation. Conventional (which includes both chemical and biological) treatment of wastewater may not be enough to reduce the microbiological concentrations to safe levels. Advanced treatment removes microbes, but at a very high cost (Lund, 1978). **Table 1-3** shows the pathogenic concentration in wastewater at the different stages of treatment according to the U.S Environmental Protection Agency (USEPA) (U.S. Environmental Protection Agency (US EPA), 1997a).

Table 1-3 - Pathogenic Contaminants at Different Wastewater Treatment Stages (U.S. Environmental Protection Agency (US EPA), 1997a)

Pathogen	Number per 100ml of effluent				Number per Gram of Sludge	
	Raw Sewage	Primary Treatment [a]	Secondary Treatment [b]	Tertiary Treatment [c]	Raw	Digested [d]
Fecal Coliform (MPN)	10^9	10^6	10^3	<2	10^7	10^6
Salmonella (MPN [E])	8,000	800	8	<2	1,800	18
Shigella (MPN)	1,000	100	1	<2	220	3
Enteric virus (PFU [F])	50,000	15,000	1,500	0.002	1,400	210
Helminth ova	800	80	0.08	<0.08	30	10
Giardia lamblia cysts	10,000	5,000	1,500	3	140	43

[a] Mainly suspended solids removal
[b] Biological treatment to remove dissolved organic matter
[c] Post-secondary, and includes coagulation, sedimentation, filtration and disinfection
[d] Mesophilic anaerobic digestion
[E] MPN= Most Probable Number
[F] PFU= Plague Forming Units

1.4 Standards and Guidelines for Wastewater Reuse in Agriculture

National and international guidelines have been published for the safe reuse of wastewater with the goal of minimizing exposure of the public to any health risks. In keeping with the acute nature of health problems associated with biological contamination, all published guidelines focus on limits on microbiological substances, especially indicator organisms such as fecal coliforms. The United Nations World Health Organization (UN-WHO) first provided guidelines for the safe use of wastewater in agriculture in 1989 (UN-WHO, 1989). These were revised in 2000 and most recently in 2006 (UN-WHO, 2000; 2006). A summary of these guidelines is presented in Appendix I along with similar regulations from several states in the USA (U.S. EP|A, 2004).

Guidelines in Egypt

In Egypt, there are multiple ministries and institutions that are involved in wastewater management and reuse in Egypt, and that have helped in providing the regulations existing today relating to the use of treated wastewater in irrigation, and some of these institutions are:

- The Ministry of Land Reclamation and Agriculture, which typically manages agricultural aspects.
- The Ministry of Housing Utilities and Urban Communities, which is involved in the domain of planning and construction of municipal wastewater treatment plants.

- The Ministry of Health and Population undertakes the responsibility of the sampling and analysis of wastewater effluents. Additionally, the ministry is therefore in charge of establishing the water and wastewater quality standards and regulations for safe use.
- The Ministry of Water Resources and Irrigation allocates water for reclamation areas.
- The Ministry of the Environment and the Egyptian Environmental Affairs Agency (EEAA) accommodates for all related environmental aspects.
- Scientific institutions and universities conduct basic and applied research activities.

The following laws and decrees are particularly related to the issue of both disposal and reuse of wastewater (Allam & Allam, 2007):

- **Law 93/1962** "regulates wastewater disposal and designates the responsibility of constructing public wastewater systems to the Ministry of Housing which is also responsible for issuance of permits regulating wastewater discharge into public sewerage networks or into the environment. The Ministry of Health determines the regulatory standards".
- **Decree No. 649/1962 and Decree No. 9/1989: Decree No. 649/1962 of the Minister of Housing issues the executive regulations of Law 93/1962**. "It specifies regulatory standards for wastewater disposal. It was updated in 1989 by Decree No. 9/1989 in which a distinction was made between wastewater disposal on sandy soils and clay silt soils. Primary treatment was set as a minimum treatment level required before final discharge. Reuse of effluent in the irrigation of vegetables, fruits or any other crops eaten uncooked is strictly prohibited. The same restriction is imposed on grazing of animals or milking cattle on the fields irrigated with wastewater. In 1995 an amendment specified the minimum degree required for wastewater treatment for the various reuse aspects. Tertiary treatment was set as prerequisite for unrestricted irrigation of crops eaten uncooked. Secondary treated effluents may be reused for irrigating palm trees, cotton flux, jute, cereals, forage crops, flower nurseries and thermally processed vegetables and fruits".
- **Law 48/1982** was" passed for the protection of the River Nile and watercourses from pollution".
- **Decree 8/1983** "is an executive regulation of Law 48/1982 issued by the Minister of Irrigation. Discharges to the Nile, canals, drains and groundwater are to be controlled through licensing. The Ministry of Health is entrusted with setting standards and monitoring the quality of discharges. Water quality standards are specified for the River Nile, treated industrial effluent to the Nile and canals, treated industrial and sanitary water discharge to drains, lakes and ponds, treated discharge from river vessels to the Nile and canals and drain waters to be mixed with the Nile or canals".
- **Law 4 of 1994**—"Environmental Framework Law by the Minister of State for Environmental Affairs (MSEA). All facilities discharging to surface water are required to obtain a license and maintain a

register indicating the impact of activities on the environment. The register should include data on emissions, efficiency and outflow from treatment units and periodic measurements".

- **Decree No. 603/ 2002**—"Decision of the Deputy Prime Minister and Minister of Agriculture and Land Reclamation for the restriction of the use of wastewater in the agricultural sector. It prohibits the use of wastewater, whether treated or untreated, for irrigating traditional field crops. Irrigation is only used in the limited cultivation of trees for timber and ornamental trees, taking into account the measures to protect the health of workers in agriculture when using this type of water".
- **Decree No. 1038/2009**—"Decision of the Minister of Agriculture and Land Reclamation to prohibit the use of wastewater, whether treated or untreated, for the irrigation of all food crops. No permission to own new lands would be approved, unless the Ministry of Water Resources and Irrigation (MWRI) confirmed the existence and suitability of a source of irrigation".

Table 1-4 lists the guidelines that are considered by practitioners as the framework for the reuse of treated wastewater in Egypt. The definitions of "primary", "secondary", and "advanced" (or tertiary) treatment in Table 1-4 coincide with the guidelines given in the 1995 Amendment to Decree 9/1989 noted previously.

Table 1-4 - Egyptian Wastewater Reuse Guidelines (The Egyptian Code for the Use of Treated Wastewater in Agriculture, 2005)

S. No	Particulates	Unit	First Group: Water Treated Primarily	Second Group: Water Treated Secondarily	Third group: Group Advanced Treated Water
1	Absorbent Bio-Oxygen (BOD_5)	Part in a Million	300	40	20
2	Consumed Chemical Oxygen COD (Dichromate)	Part in a Million	600	80	40
3	Solid Suspended matter (TSS)	Part in a Million	350	40	20
4	Oils and lubrications	Part in a Million	Non determined	10	5
5	Number of Enteric Nematoda cells or eggs	Number/ L.	5	1	1
6	**Number of Faecal Coliform Cells**	**Per each 100/mL**	**Non-determined**	**1000**	**100**
7	Maximum concentration of total solute salts "according to the degree of plant endurance"	Part in a Million	Up to 2500	Up to 2000	Up to 2000
8	Percentage of sodium absorption (permeability according to type of soil and plant)	Percentage %	25	20	20
9	Concentration of chlorides	Part in a Million	Up to 350	300	300
10	Concentration of Boron	Part in a Million	Up to 5	Up to 3	Up to 3

1.5 Problem Statement

Expanding population growth and development and associated water demand and the depletion of high-quality fresh water supplies, especially in rural and poor communities, is increasing the pressure on existing water resources. When combined with the expanding deterioration of fresh water resources due to pollution by unsustainable water management, the result is water stress and even water scarcity in arid and semiarid regions. The UN FAO Water Unit (FAO Water, 2010) reports that the use of water has been rising at a rate that is twice as much as the population growth in the last century, and, despite the fact that there are no significant signs of a global water crisis, there is no question that there is a growing number of provinces that are suffering from a chronic shortage of water. Statistics predicts that "**by 2025, 1,800 million people will be living in countries or regions with absolute water scarcity,"** and it is the arid and semiarid regions that will be most affected due to their climate conditions, population growth, and economic development (FAO Water, 2010).

This makes the reclamation of wastewater and its reuse in irrigation a favorable application that can enhance agricultural production while playing an important role in satisfying the large water demand for irrigation in arid countries. On the other hand, the contamination of reclaimed wastewater by hazardous pathogens can pose many risks to human health and the environment if it is improperly reused in irrigation or insufficiently treated. Proper evaluation of these risks requires quantitative assessment of the survival and transport of the residual pathogens from reclaimed wastewater in irrigated soil.

Many studies have shown that microbial pollutants such as viruses and bacteria may survive and even grow depending on the characteristics of the soil, the existence of food and moisture, and the temperature. A study was conducted by a team from the Academy of Scientific Research and Technology, the Ministry of Health, and the US Agency for International Development (1995) to prepare an environmental impact assessment a project for utilization of the effluent from Helwan wastewater treatment plant for cultivation of 17,500 acres of desert. Investigations of sewage plant workers and farm workers involved revealed that (Hendy, 2006): (1) 38.2% had parasitic infections (*Ascaris*, *Entamoeba Histolytica* and *Giradia*); (2) 18.4% were anemic; and (3) 27% had gastrointestinal symptoms (Dysentry and enteritis).

The application of natural fertilizers (manure) can enhance the solid phase organic fraction of the soil and considerably alter the growth and transport of bacteria in the soil column during the course of irrigation. Therefore, even if the pathogenic concentration in wastewater for reuse is satisfactory according to the regulations, site specific conditions may lead to the increase in concentration of these pathogens to levels that incur unacceptable risks to humans and the environment.

1.6 Objectives

Previous studies revealed that there is a direct relationship between the transport of *E- coli* (a fecal pollution indicator) and irrigation conditions, such as the hydraulic loading rate, and soil properties including the organic fraction of the soil (Smith & Hegazy, E. coli Transport in Soil Columns: Implications for Reuse of Treated Wastewater in Irrigation., 2006; Smith & Badawy, Modeling E. coli Transport in Soil Columns: Simulation of Wastewater Reuse in Agriculture., 2008). This thesis is a continuation, in part, of these two previous researches, but with a focus on an aspect that was identified but not fully studied in those prior efforts; namely, the impact of site-specific parameters on the growth and survival of pathogens in soil, an important consideration for the case of wastewater reuse in irrigation. Accordingly, the main purpose of this work is to study the growth and survival of an indicator bacterium (*Escherichia coli or E-coli*) under different conditions in a sandy soil, which is particularly selected since it is typical of most soils in the Middle East. The specific objectives of this study can be described as follows:

- To study the effect of varying soil organic content on the growth and survival of *E-coli* in the test soil.
- To study the growth and survival of the bacteria using different initial concentrations of *E-coli* in the water applied to the soil, corresponding to the use of reclaimed wastewater with different concentrations of pathogenic contamination.
- To study the impact of seasonal weather conditions, *i.e.*, the effects of temperature, on the survival and growth of the indicator bacteria in the soil.

To achieve these objectives, an experimental study was carried out on soil columns of the same depth and with known organic contents. The test columns were exposed to ambient atmosphere and bacterial solutions of known initial concentrations. The methods of data collection and analysis were informed by a review of selected literature that is summarized in Chapter 2, and are explained in detail in Chapter 3. Presentation of the experimental results and analysis is given in Chapter 4.

The bacteria concentration profiles in the soil columns over time should indicate the pattern of survival of the bacteria and their trend of transport for the given conditions. The results should aid in the development of sustainable practices for the cultivation of the deserts using treated wastewater instead of depending on virgin water In particular, the results should help to optimize irrigation scheduling to minimize risks to human health and the environment in addition to providing data to calculate those risks. The results should also aid in determining more realistic guidelines for acceptable levels of pathogens in treated wastewater to be used in irrigation in the MENA because they account for site-specific variables unique to the region.

2 Literature Review

2.1 Risks Associated with Using Wastewater in Irrigation

The growing need for water resources for used in agriculture calls for the application of the cradle-to-cradle sustainability concept in the application of wastewater treatment and reuse. However, if the wastewater is not treated well enough or used in its raw state, the practice can pose risks on the soil, the agricultural produce, and public health risks. Many researches have shown that these risks are correlated and their occurrence frequent. They include the following: (1) the risks of soil properties changing due to contamination as a result of using raw or partially treated wastewater; (2) the risk of negative effects on plant growth due to contamination; (3) the public health risk of harvesting and consuming contaminated crops as a result of using contaminated wastewater; (4) public health risks from long exposure to contaminated wastewater or irrigated soil; and (5) risk of groundwater contamination as a result of transport of microbes or other contaminants through the pores of the soil.

2.1.1 Risk of Soil Deterioration

Despite the existence of several standards and regulations that are based on viable scientific studies concerning the safe reuse of wastewater in agriculture, additional studies are needed that address the survival, growth, and transport of pathogenic microbial contaminants in the soil, as well as the allowable limits of contamination (Santamar & Toranzos, 2003).

One of the important concerns in the long-term reuse of reclaimed wastewater and grey water irrigation is the possibility of change in the soil properties and structure due to the presence of toxic and hazardous contaminants. The amount of elements such as calcium (Ca), magnesium (Mg), and sodium (Na) in the soil are used to calculate a property of the soil called the sodium adsorption ratio (SAR). SAR is a measure of Na concentration in the soil relative to the concentrations of Mg and Ca. If the sodium in the soil is excessive, it can damage the soil structure by reducing its water transmission and infiltration (Mace & Amrhein, 2001). Each type of soil is given a certain SAR range according to the type of irrigation water used (with specific electrical conductivity value EC, which is a measure of salinity). If the soil SAR value happens to be lower or higher than the specified range for the soil and for the irrigation water used, soil problems can occur (Travis, Wiel-Shafran, Weisbrod, Adar, & Gross, 2010) such as dispersion, swelling, and/or crusting (Varvel, Koenig, & Ulery, 2009). The concentration of Na can vary according to the type and quality of reclaimed water, and several types may contain large amounts of sodium (such as those containing laundry and dishwashing cleansers). After a long-term use of these types of grey water, sodium can be accumulated in the soil; thus, leading to structural damage.

Another important soil parameter that can be affected by the long-term use of reclaimed wastewater is the hydraulic conductivity of the soil. A case study conducted in Sicily (2007) on the effects of application of reclaimed wastewater on soil showed that at higher pressure head, there is an obvious decrease in the water retention as well as in the hydraulic conductivity of the soil in comparison to the initial values before the application of wastewater (Aiello, Cirelli, & Consoli, 2007). The suggested cause of such decrease is the change in the pore distribution of the soil (Aiello, Cirelli, & Consoli, 2007). Moreover, in the same study, bacteriological contamination tests on soil samples taken near the source of application indicated the presence of *Escherichia Coli*, *Fecal Streptococci* (FS), and *Salmonella*. These pathogens were found within a soil column at relatively large depths (0.1 m - 0.4 m) with significant concentrations measured in MPN/100 mL (Aiello, Cirelli, & Consoli, 2007). The study reported that the concentration of the *E.coli* found in the soil samples subsequent to wastewater application was higher at a depth of 0.1 m (3×10^3 MPN/100 mL), and decreased in the order of 3 log units at a depth of 0.4 m. The concentration of the FS were found to be an average concentration of 12×10^3 MPN/100 mL at a depth of 0.1 m, and scattered within the same range all through the soil column until a depth of 0.4 m (Aiello, Cirelli, & Consoli, 2007).

To summarize, the presence of sodium in high concentrations can affect the soil structure, hindering important processes required for plant growth such as aeration as well as water transmission. Moreover, these alterations may impact the accumulations and transport of toxic chemicals, heavy metals, *and* biological contaminants to an extent not accounted for in typical wastewater reuse guidelines.

2.1.2 Soil Contamination and Plant Growth

The use of reclaimed wastewater in irrigation poses a risk of affecting the microbiological activity in the soil rhizosphere, which in turn affects the transpiration process of plants as well as cause the degradation of necessary surfactants in the soil (Garland, Levine, Yorio, Adams, & Cool, 2000). The type of microbial population and the dynamics of its survival and growth as well as the vegetation composition are important variables affecting the rate of surfactant degradation in the soil (Pinto, Maheshwari, & Grewal, 2010).

There is also a potential risk of increase of soil alkalinity resulting from long-term reuse of reclaimed wastewater (Pinto, Maheshwari, & Grewal, 2010). This is particularly common when the pH of the reclaimed waste water exceeds 8. The increase in the soil pH can in turn decrease the quantity of some necessary micro-nutrients in the soil that are needed by the plants to grow (Pinto, Maheshwari, & Grewal, 2010).

There is no solid evidence that indicates the negative influence of irrigation with reclaimed wastewater in comparison with irrigation with potable water. In fact, different studies addressing this issue have shown somewhat conflicting results. For instance, one study showed that there was a noticeable increase in the nitrogen (N) and phosphorous (P) contents in plant leaf tissues irrigated with reclaimed wastewater than the contents of these very same elements on the same plant leaf tissues when irrigated with potable water. With other elements such as potassium (K), calcium (Ca), and magnesium (Mg), however, differences between the two scenarios were negligible (Manas, Castro, & De Las Heras, 2009). Similarly, the amount of cadmium (Cd), aluminum (Al), and nickel (Ni) was found to be much higher after 3 years irrigation with reclaimed wastewater than the amount of the same heavy metals after 3 years irrigation with potable water. On the other hand, there was no noticeable difference in the amounts of arsenic (As), chromium (Cr), iron (Fe), manganese (Mn), lead (Pb), and zinc (Zn) in the two scenarios. As for plant growth, measured parameters such as height, diameter, dry and fresh weight, showed that these parameters were higher in the plants irrigated with reclaimed wastewater (Manas, Castro, & De Las Heras, 2009). Nonetheless, it is always preferable to use the reclaimed water for irrigation of plants for which the yield is not directly ingested by the public, especially if the toxicity is still in question.

Another recent study addressing this issue showed (through statistical analysis) that although the dry biomass of the leaf and root measured subsequent to irrigating with reclaimed wastewater was not very different from that irrigated with potable water, there was still a minor decrease in the leaf and root biomass following irrigation entirely with reclaimed wastewater (Pinto, Maheshwari, & Grewal, 2010) compared to the biomass for the application of other treatments and mixtures. On the other hand, the same study also illustrated that the volume of the root was highest when a mixture of potable and grey water was used compared to the lowest volume obtained with the use of potable water only (Pinto, Maheshwari, & Grewal, 2010).

Several previous studies indicated adverse effects of the use of greywater on plants (Bubenheim, Wignarajah, Berry, & Wydeven, 1997; Wiel-Shafran, Ronen, Weisbrod, Adar, & Gross, 2006). For instance, findings from one study that focused on the growth of lettuce plants in soil medium indicated the occurrence of chlorises after 30 days (Wiel-Shafran, Ronen, Weisbrod, Adar, & Gross, 2006; Pinto, Maheshwari, & Grewal, 2010). In another study where growth was studied in liquid medium and Igepon-42, findings indicated the occurrence of toxicity that was observable in the browned color of the lettuce plant roots within a period of 4 – 6 hours, followed by the clampdown of the dry mass of the root after 24 hours (Bubenheim, Wignarajah, Berry, & Wydeven, 1997; Pinto, Maheshwari, & Grewal, 2010).

2.1.3 Health Risk from Agricultural Products

The transfer of contamination from soil to the yield crops can occur from various causes: (1) as a result of irrigation with reclaimed wastewater; (2) due to the use of contaminated raw manure to increase the soil fertility; (3) or even from the transport of hazardous contaminants to groundwater when the soil is saturated during irrigation. In addition to microbial contaminants, there is also the possibility of contamination with hazardous substances in the reclaimed waste water such as suspended and dissolved solids (chlorides, nitrates, sodium, boron, and heavy metals) as well as added salts during secondary and tertiary treatment processes. But the most common acute health risk among of all these contaminants are the microbial contaminants (helminthes, bacteria, and viruses) (Aiello, Cirelli, & Consoli, 2007).

Contamination can be transferred to crops during growth, harvest, postharvest, management, and/or circulation, and though it is not the only means of transmission of infection, contaminated wastewater is considered an important way through which the crops can be contaminated. The highest health risk lies in the possibility of contamination of crops that are edible raw, especially when it has been irrigated with untreated or partially treated wastewater (Ibenyassine, et al., 2007). Prominent examples are tomatoes, cantaloupes, and sprouts, and they have been, on many occasions, reported to have contamination with *Salmonellosis*. Similarly, other reports linked of infections with the presence of *Escherichia coli* O157:H7 in melons, apple eider, lettuce, and radish sprouts (Ibenyassine, et al., 2007). Other crops such as coleslaw, cabbage, potatoes, radish, bean sprouts, and cucumbers were also found to have been contaminated with *Campylobacter* (Ibenyassine, et al., 2007). In regions where the demand of water increases and there is not enough rainfall to cover for these demands, such as the MENA, the use of reclaimed wastewater in irrigation becomes a necessity. Although there is no solid evidence that *directly* links the outbursts of various infections such as fevers, cholera, and typhoid to contamination of crops to the use of reclaimed wastewater in agriculture, a pattern has been observed that indicates the correlation between crop contamination and food-borne diseases (Ibenyassine, et al., 2007).

Another study indicated that microbial survival can extend for long periods of time when the soil is saturated with both water and manure (Solomon, Yaron, & Matthews, 2002). Lettuce crop was grown in soil contaminated either from supply with raw contaminated manure or irrigated with contaminated wastewater, and the findings demonstrated that the *E.coli* contamination from the soil transmitted to the edible parts of lettuce crops (Solomon, Yaron, & Matthews, 2002). Contamination did not occur merely as a result of the direct plant exposure to contamination, but also due to the transport of the microbial contaminants through the soil and to the roots of the plants (Solomon, Yaron, & Matthews, 2002). Although the researchers used bacterial indicator concentrations higher than those normally detected in

the field, this was excused in the knowledge that even low concentrations of *E.coli* in the soil and the crops are considered hazardous to public health, for instance, at concentration of 1000 CFU/100 mL (Solomon, Yaron, & Matthews, 2002). Simple cleaning or disinfecting processes may not be very effective when it comes to infected yield of crops. Accordingly, this calls for caution and for following regulations when it comes to the reuse of wastewater in irrigation as well as in the application of manure.

A controlled laboratory environment was used to address the effect of application of manure-compost on carrot and onion crops (Islam, Morgan, Doyle, & Jiang, 2004). While carrot crops were found contaminated by microbes, the onion, garlic, and leek crops were highly resistant to contamination and showed antimicrobial activity (Islam, Morgan, Doyle, & Jiang, 2004). It is because of this very property in the family of onion crops that oil and other liquid extracts from onions are frequently used in the making of antibiotics and antimicrobial proteins such as Ace-AMP1, which was found to be highly repressive even at very low concentrations against hazardous microbes (Islam, Morgan, Doyle, & Jiang, 2004).

According to findings from an earlier study by Johnson and Vaughn (Johnson & Vaughn, 1969), reconstituted and dehydrated garlic and onion were used at concentrations of 5% and 1% (weight/volume) were repressive to *Salmonella* as well as *E-coli*, and the highest death rates were observed at concentration 10% and 5%. A similar study also showed that extracts from the Allium Genus plants can be used to prevent the growth of *E-Coli*, *Pseudomonas psyocyaneus, Salmonella Typhimurium, and Bacillus cereus* (Islam, Morgan, Doyle, & Jiang, 2004).

2.1.4 Health Risk from Exposure to Irrigated Areas

Exposure to contamination poses a definite risk on public health, especially due to the ability of microbes to transport and survive through various media and adapt to diverse conditions. They can cause diseased through their transport through skin tissues and cells, or they can migrate to the internal organs by ingestion of contaminated crops or soil. In all cases, their transport is associated with diseases and problematic infections. The most common infections reported occurring due to microbial transport from contaminated wastewater are skin diseases and intestinal diseases.

When it comes to long exposure to contaminated fields due to occupational obligations, infections from Helminthes, particularly Ascaris and Hookworms, are more important than bacterial and viral health risks or infections from protozoa, and this is perhaps owing to their higher frequency of occurrence . (Abaidoo, Keraita, Drechsel, Dissanayake, & Maxwell, 2010). The groups of individuals most at risks are therefore field workers, who are exposed to the contaminated soil the longest. One Study indicated that the

frequency of occurrence of such infections in Haroonabad, Pakistan, due to the recurrent use of reclaimed wastewater for irrigation and long exposure to contamination, can reach 80% (Abaidoo, Keraita, Drechsel, Dissanayake, & Maxwell, 2010; Van der Hoek, et al., Urban Wastewater: A Valuable Resource for Agriculture, 2002). Other recent studies indicated the emergence of skin diseases as well as intestinal diseases among farmers in Vietnam using reclaimed wastewater for irrigation (Abaidoo, Keraita, Drechsel, Dissanayake, & Maxwell, 2010; Trang, Hien, Mølbak, Cam, & Dalsgaard, Epidemiology and Aetiology of Diarrhoeal Diseases in Adults Engaged in Wastewater-Fed Agriculture and Aquaculture in Hanoi, 2007a). Nonetheless, the major problem is that in these regions, there is not enough awareness or understanding about the issue and the hazards connected with direct contact and long exposure to contaminated wastewater. Therefore, the farmers working in those fields do not link the occurrence of these infections to the use of the contaminated wastewater (Abaidoo, Keraita, Drechsel, Dissanayake, & Maxwell, 2010).

Similar studies have also shown that skin infections that frequently occur in Vietnam and Cambodia such as dermatitis (eczema) are directly linked to the use of contaminated wastewater in agriculture (Abaidoo, Keraita, Drechsel, Dissanayake, & Maxwell, 2010; Trang, Hien, Mølbak, Cam, & Dalsgaard, Skin Disease Among Farmers Using Wastewater in Rice Cultivation in Nam Dinh, Vietnam, 2007b; Van der Hoek, et al., Skin Diseases Among People Using Urban Wastewater in Phnom Penh, 2005), whereas in Katmandu Valley, Nepale, more than 50% of the farmers have reported that they were having skin problems and they too were using reclaimed wastewater for irrigation (Abaidoo, Keraita, Drechsel, Dissanayake, & Maxwell, 2010). The symptoms associated with these skin infections include itching, hands and feet swellings, and nail problems such as koilonychias (Abaidoo, Keraita, Drechsel, Dissanayake, & Maxwell, 2010). Additionally, other diseases such as anemia have been reported and are specifically associated with infections from hookworms that in turn cause nail problems as well (Van der Hoek, et al., Urban Wastewater: A Valuable Resource for Agriculture, 2002). Similar problems have been observed by farmers working in rice fields along the Musi River in Hyderabad, Pakistan, as well as famers working in vegetable fields in Ghana that are irrigated with reclaimed wastewater (Abaidoo, Keraita, Drechsel, Dissanayake, & Maxwell, 2010).

2.1.5 Potential of Contaminant Leaching to Groundwater through the Soil

The ability of microbes to leach through the soil depends on many factors (soil properties and profile, temperature, organic content, *etc*.), that will be discussed in more detail in the following sections, but a major factor is the saturation state of the soil. Microbes tend to move more rapidly and easily through the soil when it is saturated due to microbial adsorption to the soil particles, with the rate of transport

depending on the size and orientation of the soil grains (Santamar & Toranzos, 2003). In case of rainfall, the pathogens become more scattered throughout the region and the along the soil either by means of runoff from locations where manure or fertilizers have been used or by leaching and transporting through the soil column (Santamar & Toranzos, 2003). This poses a risk of groundwater microbial contamination and is highest during excessive rainfall, in which case the soil becomes saturated and contaminants in the soil leach to the groundwater. A previous study showed that both deep (153.3 m) and shallow (9.4 m) wells have been found contaminated with coliforms following heavy rainfalls (Gerba & Bitton, Microbial Pollutants: their Survival and Transport Pattern to Groundwater, 1984).

Studies of viral transport from septic tanks to groundwater as well as surface water indicated that viruses could be found up to a distance of 50 m from the septic tank in silt loam type soil, and they were also detected after 71 days at a nearby lake (Santamar & Toranzos, 2003). In another study at a farm where sludge from anaerobic digestion had been applied for 7 years, viral indicators were found at locations along several depths inside the soil and across the soil indicating the viruses' ability to transport through the soil vertically and horizontally (Santamar & Toranzos, 2003; Straub, Pepper, & Gerba, 1992; Gerba, Transport and Fate of Viruses in Soil: Field Studies, 1987).

In addition to microbiological contaminants, the risk of groundwater contamination can also be attributed to the leaching of toxic chemicals substances from wastewater applied to the soil if used in its raw form or if insufficiently treated. Potentially hazardous elements that pose public health risk include heavy metals such as Cd, copper (Cu), Pb, selenium, and Zn that can still be present in treated wastewater (Pinto, Maheshwari, & Grewal, 2010). Moreover, application of nutrients or fertilizers to the soil along with wastewater at a time when the plants need such nutrients the least can cause the leaching of these nutrients (nitrates in particular) through the soil, contaminating the groundwater (Pinto, Maheshwari, & Grewal, 2010).

2.2 Pathogen Growth and Survival in Soil

Many pathogenic contaminants, whether from applied contaminated irrigation water or from the application of fresh animal manure, can survive for long periods in soil and water through which they can also become a possible hazard by contamination of the expected plantation yield. Recent studies have shown that hazardous pathogens can enter plants through their roots and then grow and survive, regardless of the plant type. Accordingly, raw sewage has to be treated up to several orders of magnitude depending on its degree of contamination before it can be applied in irrigation to the soil or even discharged into water systems.

Table 2-1 illustrates that, depending on the degree of treatment of wastewater prior to application and the type of microorganisms present, there is a direct relationship between the transport of these microorganisms and the soil type and its physical properties. **Table 2-2** similarly shows data related to the transport of viruses from wastewater introduced to the soil. The distance to which the microbes are able to migrate in the soil can be determined through tests at several depths, and the maximum distance travelled along with the duration to travel in the soil greatly depends on the extent of contamination of the applied wastewater. Fecal coliforms, the dominant microorganisms found in secondary treated sewage, can migrate up to a distance of nearly 10 meters in fine loamy sand to gravel soil types. Such a distance only decreases to 6 meters with the application tertiary treatment containing only coliforms, which is still a large distance in which the coliforms can be found in the soil column. Furthermore, the time of travel of the pathogens in the soil can range between a few hours to several weeks depending not only on the type and extent of contamination of the water applied to the soil but also on the conditions of the host environment. Accordingly, examination of pathogenic contaminants in soil columns of varying lengths and time frames is a useful tool for assessing the reuse of wastewater in irrigation.

Table 2-1 Movement of Bacteria from Wastewater into and through Soil (Pettygrove & Asano, 1985)

Nature of Fluid	Organisms	Media	Maximum distance Traveled	Time of Travel
Tertiary treated wastewater	Coliforms	Fine to medium sand	6.1 m	--
Secondary sewage effluent on percolation beds	Fecal Coliforms	Fine loamy sand to gravel	9.1 m	--
Primary sewage in infiltration beds	Fecal Streptococci	Silty sand and gravel	183 m	--
Inoculated water and diluted sewage injected subsurface	Bacillus Stearothermophilis	Crystalline bedrock	28.7 m	24-30 hr.
Sewage in buried latrine intersecting groundwater	Bacillus Coli	Sand and sandy clay	10.7 m	8 weeks
Canal water in infiltration basins	Escherichia Coli	Sand dunes	3.1 m	--

Table 2-2 Movement of Viruses from Wastewater into and through Soil (Pettygrove & Asano, 1985)

Virus Type	Nature of Fluid	Nature of Medium	Flow Rate	Distance of Travel	Percentage of Removal
T1, T2, f2	Distilled water with added salts	9 types of soils from California	0.078 to 0.313 mL/min	45 to 50 cm	>99
Poliovirus 1	Distilled water, 10^{-5} N Ca and Mg salts	Dune Sand	1 to 2 mL/min	20 cm	99.8 to 99.9
Poliovirus 2	Distilled Water	Low humic latersols	100 to 140 gal/day.ft^2	1.5 to 6 in.	96 to 99.3
Poliovirus 2	Secondary Effluent	Sandy Gravel	--	60 m	100
Coxscackie	Spring Water	Garden Soils	--	36 in.	50
T4	Distilled water	Low humic latersols	100 to 140 gal/day.ft^2	1.5 to 6 in.	100
T7	Secondary treatment	Sandy forest	--	19.5 cm	99.6
Indigenous enteric viruses	Secondary effluent	Loamy sand soil	Intermittent Ave.: 0.02 cm/min	3 to 9 m	100

There are several factors that can affect the survival and growth of pathogenic contaminants in soil, and these factors are related to the physical, chemical, and biological properties of the soil such as the soil texture, the particle size distribution, the organic matter type and content, the pH of the soil, the pore size distribution, and the soil bulk density. There are also the properties of the host environment for the pathogens such as the temperature of the atmosphere or the intensity of solar radiation as well as the water content and the water flux in the soil. There are also other chemical and biological characteristics of the wastewater applied for such as ionic strength of solution, the pH of infiltrating water, the nature of organic matter in waste effluent solution (both concentration and size), and the type of microorganism existing in the wastewater or the extent of contamination of the wastewater as well as the density and dimensions of the microorganism. The method of application of the wastewater to the soil relates to the soil drying between applications as well as the time or season of application.

More specifically, previous studies have shown that the following can affect both the growth and survival of the pathogens in the soil as well as their transport the most (some of these conditions will be thoroughly discussed in a later section) (Pettygrove & Asano, 1985; Badawy, 2005; Chapelle, 1993):

- Soil Organic Content, soil moisture and pH level
- Amount of suspended solids and organic matter in the soil water
- Atmospheric conditions (temperature, humidity, rainfall, and sunlight exposure)

- "Eco-competition" (presence of competitive flora and fauna lowers survival)
- Flow rate of the water introduced to the soil

The reproduction and growth of bacterial populations are typically the outcome of binary fission. This process occurs in a series of steps in which the bacterial cell elongates and expands and the cell begins to divide from inside out – producing larger populations and colonies. The overall pattern of growth of bacteria with time can be explained by a simple exponential function with bacterial concentration measured in colonies per 100mL of fluid (Chapelle, 1993):

$$C(t) = C_o e^{kt}$$

where $C(t)$ = the concentration at any time t; C_o = the initial concentration; k = the first order reaction constant; t = the time. However, the pattern of growth of the pathogens in soils is affected by numerous factors and therefore may be more complex than conveyed by the previous mathematical equation. In reality, aside from the time that the bacteria takes in its transport in the soil, the time for its growth and subsequent survival in the soil is of multiple phases rather than an infinitely exponential increase. Many studies and experiments have shown that there are typically four phases in the microbial growth cycle: a **lag phase** in which the pathogens adjust to the conditions of the media and the environment; the second is the **exponential growth phase** where the pathogens start to grow and multiply exponentially with time by and large according to the equation above; the third phase is a **stationary phase** where the pathogens start consuming the food in the media; and finally the **death phase** in which the pathogen populations decrease as the amount of food becomes exhausted and their growth ceases (Chapelle, 1993).

In summary, the magnitude and the duration of growth and survival of pathogens in soil due to irrigation by contaminated wastewater is governed by the site-specific conditions, each of which ought to be studied separately in controlled experiments in order to determine their respective impacts. Several investigations have already been conducted to find the optimum conditions for the survival of pathogens in soil upon application of contaminated wastewater. These studies have shown that, generally speaking, survival tends to increase when the soil moisture and organic content increases, and when the temperatures are not too high. However, if the soil moisture is particularly acidic or alkaline, or if there is a lot of sunlight and aggressive micro-flora, the microorganisms do not survive for long (Pettygrove & Asano, 1985).

The following table shows the typical survival durations of some microorganisms.

Table 2-3 Examples of Microbes and their Survival Duration in Soil (Pettygrove & Asano, 1985)

Organism	Survival Time (Days)
Coliforms	38
Streptococci	35 to 63
Fecal Streptococci	26 to 77
Salmonellae	15 to > 280
Salmonella typhi	1 to 120
Tubercle bacilli	> 180
Leptospira	15 to 43
Entamoeba histolytica cysts	6 to 8
Enteroviruses	8 to 175
Ascaris ova	Up to 7 years
Hookworm larvae	42
Brucella abortus	30 to 125
Q-fever organisms	148

2.3 Factors Affecting Growth and Survival of Pathogens in Soil

The soil is a media in which pathogens can live and grow, and previous research demonstrated that one gram of soil can host up to approximately 10^9 bacteria colonies. Bacteria can participate in several processes in the soil as well as those associated with plant compositions such as carbon decomposition, mineralization, nitrogen fixation, ammonification, nitrification, and denitrification (Mazhar, 2010). When the soil does get contaminated with microbial contaminants, whether through exposure to contaminated wastewater or through any other source, these pathogens can remain close to the surface or at the point from which they have originated. In most instances viable microorganisms tend to travel through the pores of the system and can possibly reach the underground water, contaminating it, or they may adsorb to the soil, lengthening their survival time. The important variables that impact the growth and survival of microorganisms in the soil, and hence their transport in the soil column, were previously summarized in Section 2.2 and can be categorized as follows:

- Soil characteristics, including the organic content;
- Atmospheric conditions, exposure to sunlight, the seasonal temperatures; and,
- Type of the organism and its characteristics, including the tendency to associate with organic matter.

2.3.1 Soil Characteristics Affecting Pathogen Transport and Survival

Porosity of the Soil

Porosity is a characteristic of the soil that distinguishes its structure and the size of the openings that exist between particles. The physical structure of the soil typically influences its tendency to sustain plant life

and allow for nutrient and water transfer. This structure can be categorized according to the relative contents of clay, silt and sand (Mazhar, 2010). For instance, soils where there is substantial clay content are most likely to be multi-soil grain aggregates with sizes ranging between millimeters to centimeters (Mazhar, 2010). The permanence of these aggregates in the soil structure may in fact be aided by bacterial growth or subsurface plant structure that aid in binding. The soil pores that result are the primary means through which bacteria may transport in the soil.

Figure 2-1 illustrates three types of pores: closed, dead end, and open-end pores. Type B can cause the entrapment of the bacteria preventing their transport, whereas Type C is a continuous open end pore that allows the bacteria to grow, multiply and transport (Mazhar, 2010).

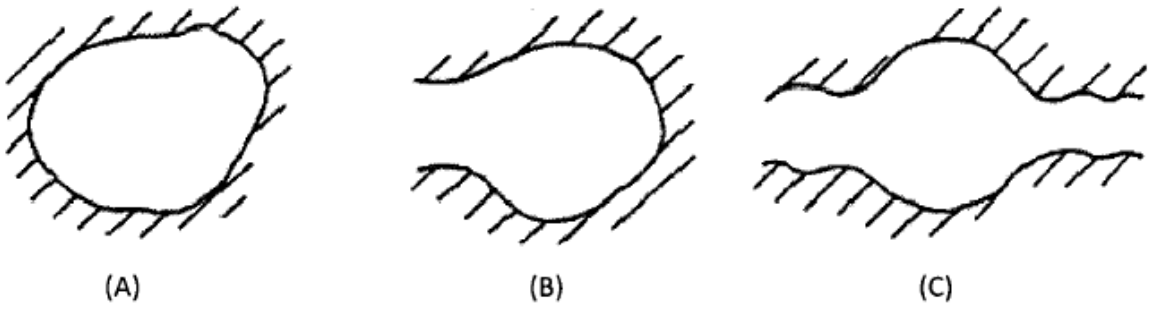

Figure 2-1 - Soil Pore Types: (A) Closed Pore; (B) Dead End Pore; and (C) Open End Pore – adapted from (Mazhar, 2010) **[**used with permission from Michigan State University by Mazhar, Mustafa A., M.S. Thesis, 2010]

Both the pores of the soil and the sizes of the pathogens that transport through these pores vary in sizes over a wide range. The sizes of the viruses, as shown in Figure 2-2, lie in the range of nanometers, whereas most bacteria lie in the range of micrometers, and protozoa in the range of hundreds of microns. The range of pore diameters for clay also lies between nanometers and micrometers, which suits the size of most bacteria, as shown in Figure 2-2. Silt and sand (macropore range) have much larger pore diameters, which makes them suitable in the transport of most types of pathogens. These sizes may be different in case of colonization of the pathogens which occurs frequently.

When it comes to the duration of pathogen survival in the soil, there is just as large a variation as that of their sizes and is greatly dependent on the host environment and its associated conditions. Although bacterial contaminants can survive for only a relatively short period of time, they reproduce and spread unevenly in colonies that they form at a great speed during this short period. Viruses, however, can only survive inside a host cell and not directly inside a host media such as soil. Some viruses, such as bacteriophages for instance, grow and reproduce inside certain bacterial cells, while other viruses that are enteric to humans can only replicate inside human cells (Buchan & Flury, 2004).

The macropore range of soil, which is also known as the drainable range for rainfall or irrigation, can be larger than 30 µm in diameter, and these pores are usually filled with air in field conditions. As shown in Figure 2-2, this makes them a suitable habitat for pathogens that can either exist in suspension within the air-water and the aqueous interface of the soil, or the pathogens can adsorb as single or colonized organisms on the surfaces of solid particles (Buchan & Flury, 2004).

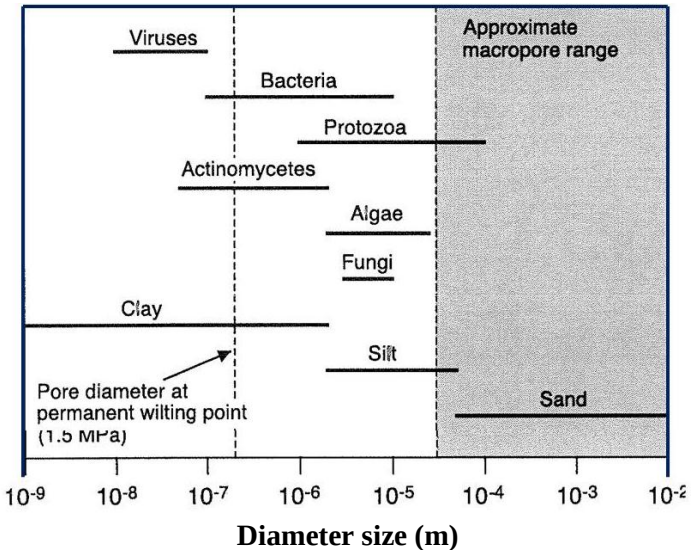

Figure 2-2 - Size variation in pathogens and soil pores – [used by permission of Marcel Dekker: *Encyclopedia of Water Science*, by Buchen and Flury, 2004]

Moisture Level and Soil Acidity

The soil moisture level indicates the amount of water present in the soil that has entered as a result of rainfall or irrigation. The water content and the pH level of the soil have been shown to affect the growth and survival of pathogens. Generally speaking, the persistence of pathogens tends to increase the most when the host medium is moist – *i.e.*, when the soil has a high water content or in seasons where there is a high frequency of rainfall. According to a World Bank Report (1980), the conditions for propagation of pathogens in feces, moist night soils, and sludge are broadly similar making the survival duration and pattern of most pathogenic contaminants in these media also similar. Table 2-4 shows that the survival duration of indicator bacteria was shown, through experimentation, to have reached a period of 5 months, whereas a microbe such as *Tubercle bacilli* can survive up to 5 years. Although these long durations do not occur frequently, they may still occur under suitable conditions in the soil.

The length of time during which the soil stays moist will also impact the duration of survival of the pathogens, and therefore is highly dependent on the capacity of the soil to retain such moisture. Such capacity is governed by the physical characteristics of the soil; namely the porosity and texture. Highly

permeable soils such as sand have very low retention capacity of water whereas the fine textured soils such as clay have a high retention capacity of water. During irrigation or rainfall, the pores of the soil are filled with both water and air. If the magnitude and the interconnectedness of the porosity of the soil is high as in sandy soils, the more water would be able to fill out these pores, but the less the capacity of the soil would be to withhold this water.

Table 2-4 Survival Times for Pathogens – Adapted from (Feachem, Bradley, Garelick, & Mara, 1980)

Pathogen	Survival Time
Enteric Viruses	Up to 5 months, but usually less than 3 months
Indicator Bacteria	***Up to 5 months, but usually less than 4 months***
Salmonellae and shigellaw	Up to 5 months, but usually less than 1 months
Vibrios	Usually less than 5 days
Tubercle bacilli	Up to 2 years, but usually less than 5 months
Protozoan Cysts	Up to 1 month, but usually less than 10 days
Helminth Ova	Varies according to species – Ascaris Ova survives only for few months.

The capacity of soil to withhold water is usually be measured in inches of water per foot of soil and can fall into four important intensities of soil moisture level indicating the extent to which the water can be reserved in the soil, namely: 1) saturation, 2) field capacity, 3) wilting point and 4) oven dry. There are typically two ways by which the water can be held in the soil, namely, by gravitational forces or through capillary action. The former occurs in first stage, or saturation, when the pores of the soil are completely filled with water and there is no air. This condition is not very favorable for pathogens that depend on both air and water, and it is also not favorable for the plants. The water detained in the soil saturation and field capacity is the gravitational water. After drainage of the gravitational water, the soil moisture content would be typically at field capacity. The water content available in this stage is for the plant use and this is the stage suitable for irrigation (Scherer, Seelig, & Franzen, 1996). At wilting point, however, the soil water content is not quite enough and the available amount may be firmly held within the small pores that the plants are unable to extract it for usage. Lastly, the "oven dry" soil occurs when all the soil water content has been removed through evaporation and the soil has completely dried out from the sun and heat. This is often the case for sandy soils as they are known for their low capacity of holding water. The following Table 2-5 shows capacity ranges for different soil types.

In the matter of survival and growth of the microbes in soil, the effect of soil moisture is not as clear as the effect of high temperature. However, experimentation showed that the bacteria in soil were most

responsive to the temperature conditions at increased moisture content of the soil. Namely, at favorable temperatures and sufficient moisture, the bacteria tend to survive for a long period of time. On the other hand, at high temperatures, the moisture in the soil could act as a catalyst in the inactivation of the bacteria in soil. This is shown in Figure 3, which indicates that the inactivation constant k reaches its maximum value (0.14) when the moisture content of the soil is highest, which may be attributed to an increase in the thermal conductivity (Dunn, Barro, & Poth, 1987).

Table 2-5 - Soils Water Holding Capacity (Scherer, Seelig and Franzen 1996)

Soil Texture	**Available Soil Moisture**
	Inches/foot
Coarse Sand and Gravel	0.2 to 0.7
Sands	0.5 to 1.1
Loamy Sands	0.7 to 1.4
Sandy Loams	1.3 to 1.8
Fine Sandy Loams	1.7 to 2.2
Loams and Silt Loams	2.0 to 2.8
Clay Loams and Silty Clay Loams	1.7 to 2.5
Silty Clays and Clays	1.6 to 2.2

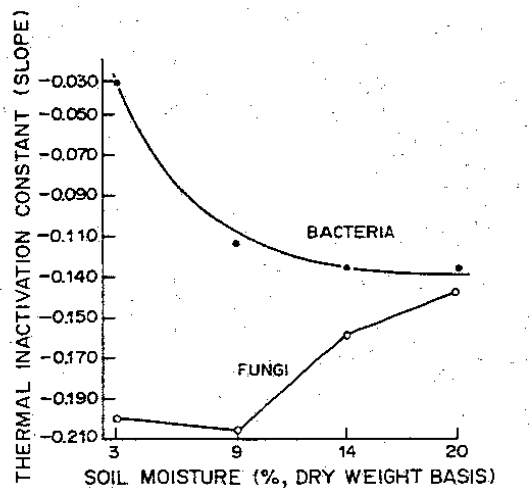

Figure 2-3 - Pathogen Survival in Response to Soil [used with permission from Elsevier Limited: Soil Biology and Microbiology. **17**(2), 1987 by Dunn et.al]

The acidity or alkalinity of the soil or the applied water is another significant condition to consider in the survival of pathogens in soil. Experimentation and tests have shown that, in general, most microbes tend to survive in alkaline media within the range of $5 < pH < 8$ rather than in acidic media with $pH < 5$. There are, however, a few exceptions to bacteria and viruses that might favor low pH media. Some of these

strains may even use sulfuric acid for instance as a source of energy. Table 2-6 shows the duration of survival in days for enteric viruses in soils according to type, moisture, pH, and temperature (Feachem, Bradley, Garelick, & Mara, 1980). Table 2-6 also indicates that the enteric viruses can survive up to a duration of 170 days (4 to 5 months) at low temperatures when the soil is alkaline, pH level is high (approximately 7.5), and with high moisture in the soil. As long as those three conditions coexist, the soil can be considered an optimum medium for pathogenic contaminants to grow as well as migrate through the soil.

Organic Matter

Organic matter is defined as anything consisting of Hydrocarbons (C-H). These compounds are usually those deriving from the breakdown of living organisms (plant, animals, insects and microbes. If the soil already contains organic matter, the source of this content can be:

- Plant or animal residues: this can be in the form of dead plants or animals, cut parts of the plants such as leaves or branches, and/or residues of existing living animals.
- Living parts of plants (*i.e.* roots)
- Pests and microbes

Table 2-6 Dependence of Survival of Enteric Viruses on pH and Soil Moisture (Feachem, Bradley, Garelick, & Mara, 1980)

Soil Type	pH	% Moisture	Temp. (°C)	Days of Survival
Sterile, sandy	7.5	10 - 20%	3 – 10	130 – 170
		10 - 20%	18 – 23	90 – 110
	5.0	10 - 20%	3 – 10	110 – 150
		10 - 20%	18 – 23	40 – 90
Non-sterile, sandy	7.5	10 - 20%	3 – 10	110 – 170
		10 - 20%	18 – 23	40 – 110
	5.0	0 - 20%	3 – 10	90 – 150
		10 - 20%	18 – 23	25 – 60
Sterile, loamy	7.5	10 - 20%	3 – 10	70 – 150
		10 - 20%	18 – 23	70 – 110
	5.0	10 - 20%	3 – 10	90 – 150
		10 - 20%	18 – 23	25 – 60
Non-sterile, loamy	7.5	10 - 20%	3 – 10	110 – 150
		10 - 20%	18 – 23	70 – 110
	5.0	10 - 20%	3 – 10	90 – 130
		10 - 20%	18 – 23	25 – 60
Non-sterile, sandy	7.5	10 - 20%	18 – 23	15 – 25

If the soil by nature contains very little content of organic matter, this may be altered by introducing organic matter as in soil-amendment practice. Such practices would not only change the chemical properties of the soil but may alter the physical and the biological properties as well. These variations in properties are very much related to the particular type of the organic matter introduced to the soil as well as the fashion by which it is being decomposed. Some of the vital impacts of variable type and amount of soil organic matter on soil-plant systems are: 1) The quantity of nutrients (*e.g*. N and P) available to plants; 2) The soil porosity, aggregation, and texture; and 3) The quantity of pathogens and organisms in the soil (Soil Organic Matter).

The mechanism by which organic matter can be utilized by the microorganisms in the soil is divided into several stages. Once incorporated with the soil, these organic materials are first broken into smaller portions by soil animals and bugs, after which they can be decomposed by microorganisms such as bacteria and fungi. The latter produce certain enzymes that further breaks down the chemical compounds of which these organic materials are made. The microorganisms then use these basic compounds as a source of energy or as nutrients for their survival and growth in the soil. The basic elements of these compounds are typically carbon, hydrogen, nitrogen, and sometimes sulfur and these elements are used by the microbes for their own growth and the development of their cell structures. (Soil Organic Matter)

Plants and other soil organisms can also use these nutrients if they are not used by the microbes first. Microbes are normally much faster in accessing and using soil-water nutrients as well as the nitrogen in the organic matter before the plants do. If the organic content does not contain an abundant quantity of nitrogen for both the organisms in the soil and the plants, the plants may eventually grow nitrogen deficient. This is the reason that makes the addition of organic content to the soil (*e.g.,* as manure or compost) necessary before agriculture, with the amount depending on the type of soil as well as other local conditions. The addition of organic material to the soil has yet another advantage and that is it can lead to the increase in the activities of earthworms that enhance the aggregation of the soil (Soil Organic Matter) . With the increase and preservation of the organic matter incorporated into the soil, there is also an increase in the amount of pathogens since the organic content becomes their source of energy for survival and multiplication. Therefore, although the addition of organic matter may indeed have a lot of advantages for agriculture especially for barren soils such as sandy soils, it might also lead to the increase of pathogenic contaminants that are also incorporated to the soil with the reuse of wastewater and a decrease in their die-out rate especially at favorable conditions. If, however, these microorganisms do die out quickly, their cells disintegrate and the nutrients that they contain can be accessible to the plants and other soil organisms (Soil Organic Matter).

2.3.2 Atmospheric Characteristics Affecting Transport and Survival

Effect of Sunlight

Prolonged exposure of soils to sunlight can make the soils dry, causing the pathogens to die out, especially those that are closer to the surface. The excess heat due to the sunlight focused on the soil as well as the exposure to ultraviolet radiation can therefore minimize the survival duration of pathogens in the soil. Such radiation not only influences the molecules of bound water in the soil, but it can also kill the cells as a result of its action on the microorganisms' nucleic acids. (Brown & Root Services Asia Pacific Pty Ltd, 2001). This is of particular importance in regions of the earth that are subject to extended exposure durations of sunlight and/or of UV radiation such as the Middle East.

Effect of Seasonal Temperatures

Researches have shown that the growth and survival of indicator pathogens is indeed dependent on the temperature. They have also shown, however, that the growth of these pathogens is not solely dependent on the temperature, but on many factors being satisfied concurrently; the most significant of these have been introduced in the previous section (sunlight and its influence on soil moisture, the nature and chemistry of the soil, and the soil- water pH). As noted previously, survival and maximum growth of most pathogens require temperatures between 10 to 25°C, wet soil that retains moisture, pH of 7.5-8, nutrients, and some shade. Extreme conditions such as those occurring in the summer and accompanied with drought and abundant sunlight are the conditions at which a maximum die-off rate for the pathogens can be noticed. Also, extreme winter conditions in which the temperatures are less than 5 degrees can also lead to an increase in the die-out rate, although the pathogens may be able to survive longer than the summer (depending on other local conditions).

The impact of seasonal effects is illustrated in **Table 2-7**. In the summer, when the temperature is highest, the survival of fecal coliforms and fecal streptococci is lowest; 24.1 hours half-life for the former and even less – 20 hours for the latter. The most favorable condition in the summer for fecal coliforms is when the pH is high (8.4), which decreases their die-off by almost half. It seems that pH is the limiting factor along with the temperature. This is clearly demonstrated by the fecal coliforms in the winter season, for even though the weather might be more favorable in the spring yet the high pH value helps the fecal coliforms survive longer in colder temperature. Also, the table shows that these conditions occur in a lagoon where it is moist almost all year long and perhaps even with enough nutrients in the soil, therefore making the pH and the season the only significant limiting conditions. With the fecal streptococci, the data support this concept more strongly than for fecal coliforms: namely how the survival of the pathogens is dependent on the season. As the data show, the most favorable conditions occur in the spring

when the half-life of the pathogens is highest (138 hours) and the die-off rate is lowest (0.12). The reverse happens in the summer where the half-life of the pathogens is lowest (20 hours) while the die-off rate is highest (0.83). This particularly happens at a field that is exposed to sunlight, and with shade the survival of pathogens increases (die-off rate decreases to 0.44 while the half-life increases to 37.8 hours).

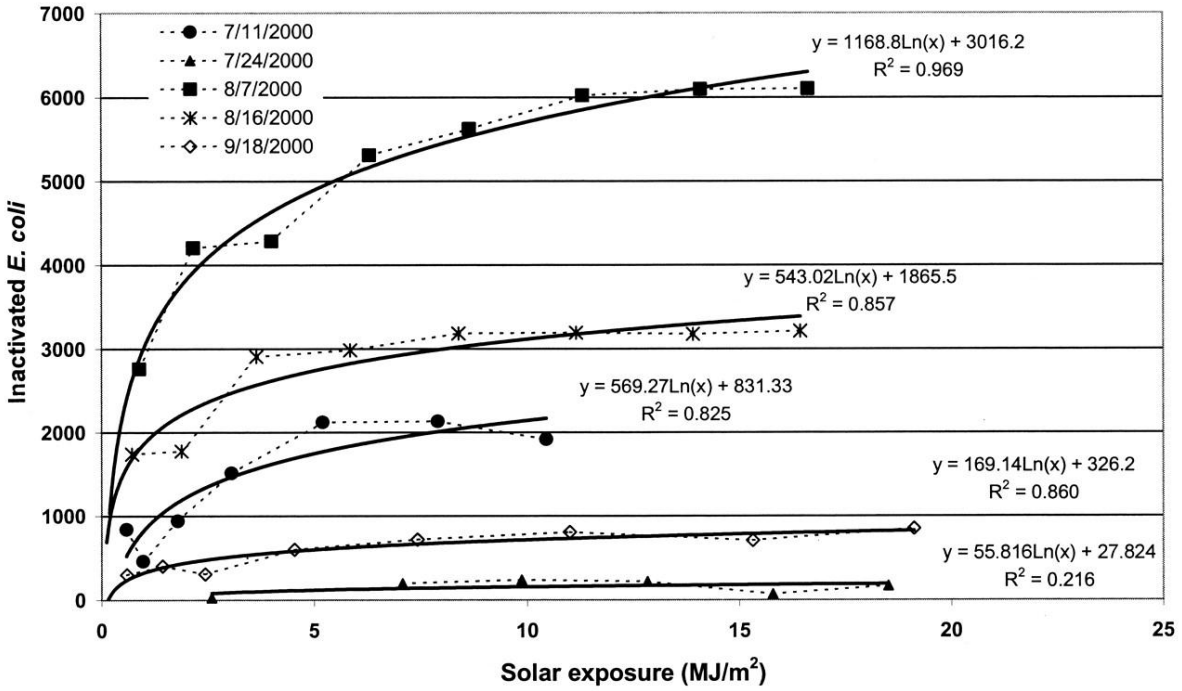

Figure 2-4 - Inactivated E-Coli vs. Solar Exposure for Different Months [used with permission from the American Society of Microbiology: *Applied and Environmental Microbiology.* **70**(7), 2004 by Whitman et al.]

Table 2-7 - Survival of Pathogens with Seasonal Changes (Reddy, Khaleel and Overcash 1981)

Pathogen	Season	First-order die-off rate k (day^{-1})	Half Life (hours)	Remarks
Fecal Coliforms	Winter	0.32	52	Lagoon, pH 7.67
		0.19	87.5	Lagoon, pH 8.03
	Spring	0.38	43.8	Lagoon, pH 7.65
		0.22	75.6	Lagoon, pH 8.16
	Summer	**0.69**	**24.1**	**Lagoon, pH 7.36**
		0.36	46.2	Lagoon, pH 8.40
Fecal streptococci	**Summer**	**0.83**	**20.0**	**Soil System – Exposed Field Site**
		0.44	37.8	Shaded Field Site
	Fall	0.16	103.9	Exposed Field Site
		0.16	103.9	Shaded Field Site
	Winter	0.35	47.5	Exposed Field Site
		0.18	92.4	Shaded Field Site
	Spring	**0.12**	**138.6**	**Exposed Field Site**
		0.12	**138.6**	**Shaded Field Site**

2.4 Effect of Manure Incorporation on Growth and Survival of Pathogens

2.4.1 Necessity of Manure Addition to Sandy Soil

One of the barriers in agriculture can be the lack of certain required characteristics in the soil needed for the plants to grow. Such characteristics can be the physical structure of the soil such as its high acidity, high permeability, and/or water retention capacity, and it may also be the chemical composition such as the availability of nutrients. In such a case, certain fertilizers need to be incorporated into the soil before plantation, and the method as well as the specific type of fertilizers ought to be chosen according to the type of the soil and the specific amendments that need to be fulfilled. Animal manure is one type of such fertilizers that have been demonstrated beneficial. The addition of animal manure to the soil increases the concentration of organic carbon (C) and decreases the runoff and soil erosion (Blanco & Lal, 2010). Several studies show that addition of animal manure is particularly needed for soils that lack the necessary nutrients for plant growth, such as nitrogen (N), phosphorous (P), and potassium (K) (Payne & Lawrence, 2011). Along with these nutrients that the animal manure can provide, it is also a highly rich source of organic matter and other important nutrients such as sulfur (S), calcium (Ca), magnesium (Mg), and zinc (Zn) that increase the potency as well as the quality of the soil (Zingore, Delve, Nyamangara, & Giller, 2008).

The most needed nutrients for the survival and growth of living organisms in soil (*e.g.*, plants and pathogens) are typically nitrogen, phosphorous, and potassium. Nitrogen in manure can either be found in the organic matter or inorganic nitrogen in the form of NH_4-N. The latter is released fast and is immediately available for plant consumption. The organic nitrogen however mineralizes gradually after which it can be available for the plants. Despite their immediate availability due to their fast release, the inorganic nitrogen can also be lost quickly because of the high volatility of ammonia. To overcome this and prevent the losses of inorganic N, the fresh manure can be directly added to the soil (Payne & Lawrence, 2011). This practice, however, is risky since it may contaminate the soil even before applying the wastewater for irrigation (Jiang, Morgan, & Doyle, 2002). The other alternative is to depend on the slow release of nitrogen available in the organic matter of the manure. Unlike nitrogen, potassium and phosphorous only exist in the manure in inorganic forms and are promptly available for plant consumption, thus making them similar to the potassium and phosphorous in commercial fertilizers (Payne & Lawrence, 2011).

The increase of organic matter in the soil can also lead to the increase of the ion exchange capacity and the metal retention capacity of the soil. The quantity and quality of the soil organic matter (SOM) added to the soil in turn affects other soil characteristics such as pH, electrical conductivity, and redox potential.

Most importantly, the SOM provides the needed food source for biological metabolism (Nieder & Benbi, 2008).

Incorporating manure is therefore regarded both economical as well as useful for amending soils that are nutrient- poor such as sandy soils (Semenov, van Overbeek, & van Bruggen, 2009). Not only does it develop the quality of the soil for agricultural use, but also it adds to it those necessary elements to enhance soil composition, and can sometimes raise the pH when the soil's acidity is high (Zingore, Delve, Nyamangara, & Giller, 2008).

2.4.2 Increase of Organic Content in Soil Due to Manure Addition

The means by which the quantity of SOM can be controlled in the soil depends on the type and the needs of the soil; the amount added to the soil has to balance the amount lost through decomposition. The SOM can therefore be increased by increasing the production of organic plant materials to be used through the application of the basic agricultural practices of irrigation, use of fertilizers, cover crops, and incorporation of animal manure in the soil; or by decreasing organic decomposition by tillage reduction or elimination (Zhang & Stiegler, 1998).

The practice of addition of animal manure to the soil, whether in solid or slurry forms, is highly beneficial as manures can be highly rich in organic content as well as other nutrients. The SOM added from solid manure is in the form of straw and coarse organic materials at first (Unc & Goss, 2004), after which they later decompose and become linked to the mineral carbon content of the soil. **Table 2-8** shows the typical constituents of different animal manures and the percentage of these constituents that should be found in ideal conditions. The lowest percentage of manure organic content exceeds 5% of the total weight – about 7% in horse manure. Therefore, the selection of the manure type to be added depends on the availability of the animals and the amount of organic content needed for the soil.

According to Blanco and Lal (2010), the increase in the concentration of the organic C in the soil is linear with the increase in the application rate of the manure. Figure 2-5 shows the depth distribution of the SOM content in the soil upon the elimination of tillage as one of the suggested practices to control the SOM. The figure clearly shows than the addition of manure can increase the quantity of the SOM considerably. The improved aggregation of the soil as a result of manure incorporation upholds the storage of this organic carbon for a long period of time (Blanco & Lal, 2010). As a result, the particulate organics as well as the organic carbon that exists in the mineral soil aggregates both increase with the addition of the manure (Blanco & Lal, 2010).

Table 2-8 - Nutrients in Different Types of Manures (Manure is an Excellent Fertilizer, 1998)

	Nitrogen (N)	Phosphorus (P_2O_5)	Potassium (K_2O)	Calcium (Ca)	Magnesium (Mg)	Organic matter	Moisture content
FRESH MANURE	%	%	%	%	%	%	%
Cattle	0.5	0.3	0.5	0.3	0.1	16.7	81.3
Sheep	0.9	0.5	0.8	0.2	0.3	30.7	64.8
Poultry	0.9	0.5	0.8	0.4	02	30.7	64.8
Horse	0.5	0.3	0.6	0.3	0.12	7.0	68.8
Swine	0.6	0.5	0.4	0.2	0.03	15.5	77.6
TREATED DRIED MANURE	%	%	%	%	%	%	%
Cattle	2.0	1.5	2.2	2.9	0.7	69.9	7.9
Sheep	1.9	1.4	2.9	3.3	0.8	53.9	11.4
Poultry	4.5	2.7	1.4	2.9	0.6	58.6	9.2

Research has also shown that without the addition of manure or fertilizers with organic matter, the SOM declines slowly (Kihanda, Warren, & Micheni, 2007). This rate of decline may be higher in mineral sandy soils with naturally low organic content than in clay soil, and such decrease in SOM tends to be furthered by continuous cultivation. A comparison between forest and planted soils in Nigeria demonstrated that the SOM of the planted soils were only half of the amount of the SOM in the forest soil. It also demonstrated that organic degradation of clay soil occurred at a rate of 0.5% to 1.5% per year, while the sandy soil organic degradation rate was from 5% to 10% per year (Kihanda, Warren, & Micheni, 2007).

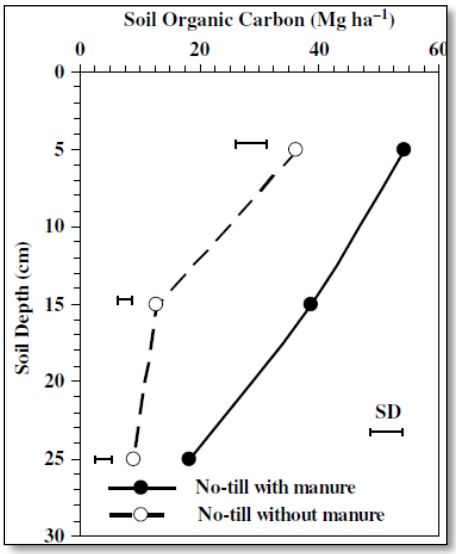

Figure 2-5 - Depth Distribution of SOM on Soil (Blanco & Lal, 2010) [used with permission from Springer Science: *Principles of Soil Conversion and Management.* 2010 by Blanco and Lal]

2.4.3 Impact of Soil Organic Content Increase on Growth and Retention of Pathogens

The survival and multiplication of pathogens in soils depends greatly on interrelated factors, among which is the availability of the soil organic matter (SOM). The SOM contains the nutrients that the microbial cells need for survival, and the increase of SOM through the addition of manure, organic waste, or fertilizers with organic content increases the retention capacity of the soil for these nutrients (Jamieson, Gordon, Sharples, Stratton, & Madani, 2002). In addition to these nutrients, the SOM increases the water retention capacity of the soil, as mentioned earlier, and becomes the main source of carbon for the microbes, making the soil a favorable media for survival and growth of enteric pathogens. Research shows, however, that the fecal coliforms may not survive if there is too much moisture in the soil (Jamieson, Gordon, Sharples, Stratton, & Madani, 2002). In such conditions, the functional organic carbon that is needed for the bacteria decreases and the die-out rate of the bacteria increases since the available organic content for their use would not be enough to cover their metabolic rate (Jamieson, Gordon, Sharples, Stratton, & Madani, 2002).

The microbial community can also increase with availability of SOM even if the conditions such as the temperature or the pH are not quite favorable. This can be attributed to the adsorption of microorganisms to the soil organic matter. The decomposed organic compounds from the manure can protect the enzymes of adsorbed cells from degradation resulting from high temperatures, low pH, or protolysis (Nieder & Benbi, 2008). Studies showed that the effects of organo-humic substances on enzymes and organic-enzyme interactions are greatly dependent on the carboxyl groups of these substances (Nieder & Benbi, 2008), which again depends on the type of manure added to the soil and the means by which it is added.

Many studies have shown that indicator bacteria persisted longer and increased with the increase of organic content in the soil (Unc & Goss, 2004). One explanation suggests that the organic matter in the soil may contain both soluble and insoluble organic carbon compounds, and they can interact with mineral as well as particulate constituents leading to the mineralization of some of the organic compounds (Bernal, Roig, Lax, & Navarro, 1992). The more and the faster the organic matter in the soil transforms into minerals, the more the soil (especially sandy soils) would contain water soluble carbon salts. This occurs more on sandy soils with larger grain sizes, where the organic matter is not as strongly adsorbed on the particles as opposed to the strong adsorption of these compounds on the fine grains of clay. It has been found in previous studies that the humic substances that are dissolved in the soil are linked mainly to hydrophobic forces due to the fact that the aggregation and reactivity is chiefly controlled by the non-polar constituents of these humic substances (Piccolo, Conte, & Cozzolino, 1999). The organic soils dissolved in the soil therefore behave in the same way as cationic surfactants (Smith, Tuck, Jaffe, &

Mueller, 1991). These substances/surfactants adsorb to cation-exchange locations in the soil, raising the hydrophobic property of the soils and in turn promoting the retention of other hydrophobic molecules in the soil (Lee, Crum, & Boyd, 1989; Smith, Tuck, Jaffe, & Mueller, 1991; Unc & Goss, 2004; Mortlandt, 1986). The retention of organic cations in the soil depends greatly on the "cation exchange capacity (CEC)" of the soil, with smaller grain size (clays) having higher CEC and therefore greater hydrophobic retention (Mortlandt, 1986; Unc & Goss, 2004).

In an unsaturated soil, the addition of manure increases the possibilities for the interaction between the organic content dissolved in the soil, and both free ions and hydrophobic molecules in the soil, which leads to an enhancement in their solubility (Smith, Tuck, Jaffe, & Mueller, 1991). Due to this increase in solubility and the increased percentage of organic content due to manure addition, hydrophobic microbes would persist in the soil and may also increase. This in turn increases the competition for attachment at organic surface sites in the soil, and such attachment is favored by hydrophobic bacterial cells associated with organic molecules (Unc & Goss, 2004). The bacteria-carbon compound complexes formed from these interactions may also have the potential to survive and grow/decay autonomously without being affected by external factors (Unc & Goss, 2004).

2.5 Effect of Temperature on Survival and Growth of Pathogens in Soil

Another important factor impacting the growth and die-out rates of pathogenic contaminants in soil is the atmospheric conditions, and the continuous changes in the temperature and rainfall in dry areas such as reclaimed deserts. Research shows that there is response in terms of the survival and growth of the microbial community and their functionality in terms of the usage of carbon substrates and enzyme activity in the soil. Such response is just as dependent on the seasonal change in temperature, the local humidity and rainfall, exposure to sunlight and/or radiation, and the consequent change in soil moisture as it is to the availability of organic content and nutrients in the soil (Bell, et al., 2009). This is shown in **Table 2-9**, which depicts the dependence of the survival duration of *E-coli* in soil on the fraction of manure added to the soil as well as the temperature. It also indicates that the most prolonged survival occurs at temperatures within the range of 15°C - 21°C.

The rates may differ from one microorganism to the other (fungi from bacteria) but there is a general trend that explains the influence of the surrounding conditions on the survival of these microorganisms in the soil. **Table 2-10** shows the results of a study inspecting the influence of different temperatures on the survival of several types of pathogens (Ehrlich, Miller, & Walker, 1970); namely, *Serratia marcescens*, *Escherichia coli*, and spores of *Bacillus subtilis var. Niger*. The most favorable temperature for these

indicator species is 24ºC as given by the negative value of the die-out rate which means the multiplication and growth of the microbial community rather than degradation with time. On the other hand, the highest die-out rate occurs at high temperatures, which usually occurs in the summers of arid landscapes.

Table 2-9 - Survival of E. coli O157:H7 in Manure-Amended Soil – Adapted from (Jiang, Morgan, & Doyle, 2002)

	Days of Survival		
	5°C	**15°C**	**21°C**
Manure: Autoclaved Soil			
1:10	77	138	103
1:25	63	>226	231
1:50	70	>226	231
1:100	35	>226	193
Manure: Un-autoclaved Soil			
1:10	42	34	103
1:25	42	152	193
1:50	56	109	174
1:100	49	109	131

Table 2-10 - Decay Rate of Bacteria at Different Temperatures – Adapted from (Ehrlich, Miller, & Walker, 1970)

Temperature (°C)	**Decay Rate (%/min)**
-29	0.19
4	0.10
10	0.04
16	0.05
24	-0.09
49	0.49

Due to the minor daytime temperature variations occurring in the summer in arid regions, the succeeding changes in the survival and growth of microbial contaminants is also minimal (van Donesl, Geldreich, & Clarke, 1967). Accordingly, not only does the summer high temperature and drought cause the die-out rate of the pathogens to be very high, but research shows that even with crude records, the die-out rate of the pathogens can have a logarithmic slope. At lower temperatures accompanied by less exposure time to sunlight and radiation, the pathogens can survive for longer times even with minimal nutrition in the soil. In such a case, variations in their growth trend can be noticed due to more variability of the surrounding conditions, each of which can influence the activation and growth of microbes (van Donesl, Geldreich, & Clarke, 1967).

The impact of ambient temperature on a soil is related to its thermal conductivity and the heat capacity. Soils that have a high thermal conductivity are heated and/or cooled faster than soils with low thermal conductivity. Conversely, soils with low heat capacity gain and lose their heat (temperature) faster than

soils with high heat capacity. These properties are determined by the particular type and constituents of the soil (porosity, SOC, minerals present, and moisture level) (Bonan, 2002).

The total thermal conductivity of soil is typically a weighted average of the thermal conductivities of its constituents: solid mineral particles, air (in the pores), and moisture. Because the air and water have much too low thermal conductivity in comparison with that for the solid mineral particles, this makes thermal conductivity of sandy soils that have low moisture level, low porosity, and high quartz content very high. Conversely, in clays, which have higher SOC and porosity, the thermal conductivity is low. The presence of SOM in a way reduces the thermal conductivity of the soils because the thermal conductivity for the organic materials is very low (Bonan, 2002). An investigation of different kinds of soil with the same bulk density is illustrated in Figure 2-6, and it shows that the thermal diffusivity is highest for sandy soils. Heat transfer downwards through soil layers results in a temperature slope that changes with the depth of the soil. (Hoeh, 2006). This vertical heat transfer downwards through the soil can be an important factor in the survival and transport of pathogens in the soil. Due to the radiation to which the soil is exposed to during the day, the pathogens at the surface do not survive and die-out at a high rate, or they transport to lower layers where the heat is much less.

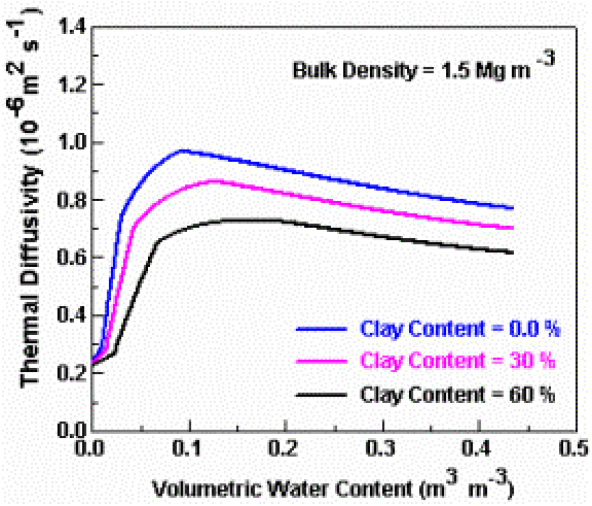

Figure 2-6 Thermal Diffusivity vs. volumetric water content for different types of soils [used with permission of Imperial College London, 2006 Thesis by Hoeh, M]

Previous studies also showed that the concentrations of bacterial cells are highest at greater depths, and this is due to several factors: One reason is the higher exposure to ultra-violet (UV) radiation on the surface layers as well as the heat from the sunlight causing the bacterial contamination on the surface to die-off more quickly and/or the transport of bacterial cells to the deeper layers of the soil. Another reason is the rapid evaporation of water content from the surface due to the longer exposure to sunlight and heat,

and also the quicker drainage of the porous sandy soils which mitigates the bacterial contaminants at the surface. Last but not least, the organic content is also decomposed more slowly with higher moisture content and less exposure to heat and oxygen (Smith & Badawy, 2010).

2.6 Selection of Indicator for the Study

According to the US EPA, if defined in the most general sense, an indicator species is that which designates contamination just through its mere presence, as well as advocates the source of this contamination (U.S. Environmental Protection Agency (US EPA), 1978). More explicitly, indicator species function as representative for other pathogenic polluting species and their existence validate contamination by enteric microbes originating from warm-blooded animals excretions (including humans), and these enteric species include a diverse amount of bacteria, viruses, and protozoa. The density (or concentration) of these indicator species is therefore used as a measure of quality and a method of assessment of safety (of water or soil).

Although several types of bacterial indicators have been used to determine the level of contamination and assess the quality as well as the safety of water depending on the type of application, there is not a specific type that is particularly and universally standardized for use by federal regulations (Noble, Moore, Leecaster, McGee, & Wiesbery, 2003). Accordingly, a specific group or a specific type of indicator can be used to indicate a certain level of contamination, according to the specific classified standards of water quality programs proposed for a specific region or country.

The most commonly identified types of microbial indicators used today are the total coliforms, the fecal coliforms, and the enterococci (Noble, Moore, Leecaster, McGee, & Wiesbery, 2003). Previous research has shown that pathogenic contamination is recurrently associated with fecal contamination (Leclerc, Mossel, Edberg, & Struijk, 2001). Accordingly, water quality control programs nowadays base their testing on fecal coliforms as indicators of fecal contamination as well as other species that pose public health risk. (Leclerc, Mossel, Edberg, & Struijk, 2001). For this study, the *Escherichia Coliform* (E-Coli), which is a subset of the fecal coliform group was selected as it is considered a very good representative of fecal contamination that poses a public health risk. Moreover, as noted in Chapter 1, virtually all international and Egyptian guidelines for reuse of treated wastewater in agriculture state limits on *E. coli* – in some cases the only biological contaminant listed – as a key standard for suitability for a given reuse water. Similarly, using *E. coli* means that the results of this study can be more readily compared and integrated to the growing number of studies worldwide on the survival and transport of coliform organisms as a result of application of wastewater to soil (Foppen and Schijven, 2006).

3 Materials and Method

3.1 Overview of the Experiments

The experiments done for this study can be divided into two main phases:

Experimentation Related to the Soil:

The soil was first tested to determine its properties such as bulk density, particle density, moisture content, pH, and Total Organic Content (TOC). Additional tests such as a sieve analysis to determine the grain sizes, and Infrared (IR) analysis to find out whether or not there are any important chemical functional groups in the soil were also performed. Other properties such as the soil porosity could be determined from the data obtained from the bulk density test and the particle density test. All of the experiments conducted in this phase were done according to the standard methods for soil analysis.

Bacteria Related Experiments:

Initially, the designated bacterial strain was reproduced from a stock culture to enable preparation of working buffer solutions at target concentrations and to know the expected stability/survival of the bacteria in solution. A concentration vs. time curve was constructed to reveal the different phases of bacteria survival (lag, growth, saturation, and death). The major effort of this research was the bacteria survival experiments in essentially static soil columns. These experiments were set up and prepared in the laboratory, but the columns were subjected to the ambient atmosphere.

3.2 Laboratory Apparatus and Set-up

For the soil column experiments to determine bacteria growth and survival, 3-inch PVC APL columns cut from drinking water grade pipes were used. Seven of these columns were used for these experiments, each containing an internal mesh filter and 1-cm long internal supporting clips cut of the same pipe as the columns. Each column was cut so as to be filled with 5 cm of soil as well as a minimum height headspace of 3 cm in addition to an extra 3 cm allowance needed for the internal mesh supporting assembly. A small section of the column clip having the same diameter of the column was cut out so that it can fit inside the internal diameter of column and at the same time can hold the mesh tightly.

The seven columns are placed, during the experiment, on wide funnels that are fixed on wooden rack as shown in **Figure 3-1**. The purpose of these racks with the funnels was to observe any excess buffer solution to pass through in case the volume of the buffer prepared was more than the pore volume needed

for the column. However, no substantive water was ever seen to drip from the columns during any of the 18 experiments. The dimensions of each of these columns are presented in **Table 3-1**.

Figure 3-1 – Soil Columns Used in Bacteria Growth and Survival Experiments

Table 3-1 - Dimensions of the Column

Column Dimensions	**Symbol**	**Value**	**Units**
Column Internal Diameter	D	6.80	cm
Column Internal Area	A	36.32	cm^2
Sand Column Height	H	5.0	cm
Sand Column Volume	V#	181.58	cm^3
Sand Column Volume (corrected for inner lip)	V	170.9458	cm^3

The supporting screen used in the soil columns is a 100-mesh of stainless steel and wire diameter of 0.10 mm. The piece of mesh cut for the column had an approximate diameter of 7 cm, and is used to hold the soil intact inside the column during the experiment and at the same time allow for any excess solution to flow out of the column. To support this mesh and prevent the occurrence of any wrinkling inside the column, the mesh rolled over a male-female adaptor (cup-shaped), after which this combined setting of the mesh and the adaptor was inserted inside the column face-up as shown in **Figure 3-2**. Internal clips were then provided to support the mesh inside the column. These internal clips were typically round

pieces of polyethylene disk of approximately 1 cm height and were punctured in the middle to provide the clipping action inside the column. Each disk has a thickness of 6 mm.

Figure 3-2 - A Column and the Mesh Inside

3.3 Equipment Sterilization

All instruments and equipment had to be thoroughly cleaned and sterilized according to their material classification prior to an experiment..

The PVC Equipment Used (Columns, Clips, and Funnels) in the Bacteria Survival Experiments:

- First, they were well washed with soap and water, then
- They were rinsed for 12 – 24 hours in a freshly prepared lab antiseptic (such as chlorinated water, which proved to be very effective in sterilizing the equipment), then
- They were rinsed in deionized distilled water (DI water) for a few minutes, then
- They were well wrapped in aluminum foil for keeping over-night.
- Right before the experiment, they were rinsed in boiling DI water for 5 minutes, then
- They were freezer cooled for 2 minutes to reach the lab temperature (23 °C)

Glassware (Test tubes, beakers, pipettes…etc.) and the Mesh Filter

- First, they were well washed with soap and water, then
- They were rinsed with DI water, then
- They were placed in the oven and/or autoclave for solid sterilization
- After they are removed, they are sealed with aluminum foil

Other Disposable Equipment Used: Petri dishes and membrane filters are manufacturer sterilized and are only used once.

3.4 Soil Characterization Experiments

3.4.1 Soil Preparation and Analysis

The soil used in all experiments was collected from a plot being used for recent plantations of ornamental plants and/or trees at the New AUC campus in New Cairo. The soil is classified as a sandy desert soil that has not yet been amended, and from the top 10-15 cm of the soil column.

1. Bulk Density:

The bulk density is a measure of how compact the soil is and how firmly it is packed to the shape of the soil beds. It can be tested by one of several methods. The most accurate measurement for the bulk density can be done if the sample is obtained directly from the field using excavation and core sampling tools, but it can also be done in the laboratory in case field measurement is not possible, as in this work.

- Use a dry empty graduated cylinder and measure and record its weight.
- Fill up the cylinder with an oven dry (dried at a temperature of $\geq 105°C$) soil sample, then
- Record the volume that soil takes on the cylinder, and the total weight of both the soil and cylinder.

The particle density can therefore be calculated from the mass of the solid particles in a specific volume according to the following equation:

$$Bulk\ density\ (\frac{g}{cm^3}) = \frac{Weight\ of\ cylinder\ and\ soil - Weight\ of\ cylinder}{Volume\ of\ soil}$$

Particle Density:

The particle density is also sometimes referred to as ‘real density’, and it is the measure of the mass of the soil in a specific volume, which is only the volume of the soil particle components excluding the volume of the pores that contain air and water. The procedures for this test are as follows:

- First a dry empty graduated glass cylinder is weighed on a balance and its weight is recorded.
- An amount of approximately 25 g of soil sample is placed inside the cylinder, and the total weight is then re-measured on the balance and recorded.
- Some water is added to the soil in the cylinder and the mixture is boiled for about 10 minutes to remove all air bubbles.
- The cylinder with its contents cools down, and is left to sit for 24 hours.
- After 24 hours, the graduated cylinder with its contents is filled with water until a 100 ml volume is reached, after which the total weight is once again measured and recorded.

The particle density can therefore be calculated from the mass of the solid particles in a specific volume according to the following equations:

- Mass of soil = Mass of soil and container – Mass of empty container (g)
- Mass of water = Mass of water, soil and container - Mass of soil and container (g)
- Volume of water = Mass of water / Density of water (cm3 or ml), where the density of water equal to 1.0 g/cm3 or g/ml
- Volume of soil = Total volume of the mixture (100 ml) – Calculated Volume of water (cm^3 or ml)

Soil particle density = Mass of soil / Volume of soil (g/cm^3)

2. Sieve Analysis

The sieve analysis is a laboratory test used on oven dry soil, and follows the following procedures:

- The sieves are stacked on top of each other in descending order of mesh opening size (largest opening on top of the stack and smallest at the bottom)
- Place an empty dry pan with known recorded weight at the bottom of the stack and this is the pan where the finest grains are collected.
- Use a balance to weigh accurately an empty dry beaker.
- The sample soil in the beaker is slowly (to avoid blinding of the openings) emptied into the stack.
- The stack is slowly and carefully shaken
- After shaking and settling, the mesh with largest opening on top of the stack is emptied in the empty beaker weighed in step 3, and a brush is used to carefully remove all of the particles from the sieve.
- The beaker with the coarsest particles inside is reweighed accurately and the weight is recorded.
- Repeat steps 6 – 9 for each of the following sieves until the bottom pan, each time including finer particles and recording the cumulative weights.

Organic Content Test

The carbon in the soil usually can be oxidized to carbon dioxide (CO_2) when the soil is subjected to a high temperature of 900 °C along with a flow of oxygen containing gas and free from CO_2. Also, this amount of heat decomposes entirely any carbonates present in the soil (Forest Soil Co-ordinating Centre, 2006). Several methods can then be used to measure the amount of carbon dioxide released: titrimetry, gravimetry, conductometry, gas chromatography, or through infrared (IR) analysis, depending on the availability of the equipment.

There are two ways through which the organic carbon content in the soil can be determined (Forest Soil Co-ordinating Centre, 2006): The first is a direct method which involves the removal of any carbonates existing in the soil by first treating the soil with hydrochloric acid (HCl). The other way is an indirect determination method in which a correction of the organic content is used for the amount of the carbonates present in the soil.

The equipment needed for this test includes an accurate balance, a spectrophotometer, an oven dried and sieved (using 2 mm mesh size) sample of soil of known carbonate content, and glassware for preparation of the calibration buffer needed for the device. The method used in this research was Hach Method # 8097 for soil and the device used in the lab is DR/2000 Spectrophotometer. For this test, the following procedures were carried out:

1. 1g of soil was first weighed and placed in a 250 ml sterilized Erlenmeyer flask.
2. 10 ml of 1N Potassium dichromate solution was the pipetted into the flask and to another empty and sterilized 250 ml Erlenmeyer flask, which was to be used as the blank.
3. A volume of 20 ml of concentrated sulfuric acid was then pipetted into each of the two flasks.
4. Each flask was then stoppered using an upturned 50 ml Erlenmeyer flask, and both flasks were then twirled slowly for mixing.
5. The flasks were then left on pads to cool for about 10 minutes.
6. A volume of 100 ml of DI water was then added to each of the flasks, and the flasks were sharply twirled for good mixing.
7. A volume of 25 ml of the soil flask mixture was next filtered into the 50 ml Erlenmeyer flask, whereas the same volume is obtained directly from the blank without filtering.
8. The blank was then used to calibrate the device and set it to a zero reading, and then the filtered solution from the soil mixture was measured using the device (after the handle is rinsed with DI and wiped to dry) and the percentage of organic content was recorded

3.4.2 Soil Preparation for the Experiments

Before it is used in any of the bacteria survival experiments, a large amount of the soil is first oven dried (105 °C) and sieved using a 10-mesh of size 2mm. The purpose of this practice is to remove coarse particles such as rocks and stones from the soil and the main reason for this is to provide the controls needed for the experiment: 1) these coarse particles may offer adsorption surfaces for the bacterial colonies other than the soil particles themselves; and 2) due to the large difference between the bulk density and the particle density of the soil, the presence of these particles may increase the pore spaces, which may in turn affect the filtration of the buffer solution through the soil.

Biological analysis was conducted on soil samples prior to sterilization and indicated that there was no significant concentration of fecal coliforms in the soil (< 300 CFU/100 ml). Also, the soil sample obtained was tested for water content and it was found to be substantially dry (5% water). Nonetheless, the soil was still placed in the oven and/or autoclave at high temperature (150°C) for drying and sterilization.

Finally, the sterilized dry soil was carefully packed into each of the sterilized columns in such a way as to make the bulk density in the column as close as possible to the previously determined value for the soils, and also care was taken during packing the soil into the column so as to avoid any soil layering or the formation of macro-pores in the layers. To do so, each 1 cm depth marked inside the column was first filled with the soil and the soil was pressed using a piston to homogenize that 1 cm layer. This latter process was repeated until all five 1-cm layers were filled entirely with soil.

3.4.3 Changing Organic Content of the Soil

To increase the organic content in the soil, horse manure (obtained from a horse farm nearby El- Haram area in Giza) was added to the soil and homogenized to minimize the variations in the bacterial densities at each 1 cm layer. The following detailed procedures were followed to prepare the soils with 0.3% and 0.5% organic content (O.C.):

1. Fresh horse manure was first dried in the oven for at least 3 hours (at 105°C) and then sieved using the standard 10-mesh with size 2 mm to remove any coarse particles and excess fibers.

2. The manure was then transferred into a glass flask, sealed well, and autoclaved for 15 – 20 minutes at 120°C in order to sterilize it before use and avoid the transfer of any bacteria transferred to the manure from the meshing process that may affect results of the experiment.

3. A small sample was taken from the manure using sterilized equipment, weighed using an accurate balance, and then added to the soil and mixed well to homogenize the distribution.

4. 3 samples were then obtained from the mixture and tested for TOC, and the results recorded.

5. If the average amount of the TOC in the soil turned out to be less than the target percentage, another small sample of the sterilized manure was taken, and steps 3-5 were repeated until the target organic content was reached.

6. If the organic content turned out to be more than the target percentage, another amount of dry sieved and sterilized soil is added and steps 3–5 are repeated until the target organic content was reached.

3.5 Bacteria Related Experiments

3.5.1 Bacteria Strain Preparation and Maintenance

Escherichia coli (E-coli) strain was utilized in these experiments having the code ATCC 25922. The original strain of bacteria was provided on an agar media plate and the strain maintained and inoculated at regular intervals (every two or three weeks). For this particular purpose, the preparation of media plates of the type MacConkey Agar (#401670, Biolife, Rome) was needed. The preparation was done according to the instructions provided by the manufacturer. For preparation of the buffer solutions of bacteria, another type of media was used namely MacConkey Broth (#401675, Biolife, Rome). This media was also used to culture the bacteria 24 hours prior to each experiment. When prepared, about 9 ml of the broth solution media was poured into three test tubes and these test tubes are closed and kept in a temperature-controlled unit at (2-4°C).

For the maintenance of the bacteria, the bacteria was introduced to a new agar media plate by means of daubing the surface of the bacteria populations from the original plate and onto the surface of the new agar plate using a sterilized steel rod. After this process, the new plate is left for 24 hours in the incubator for the bacteria to grow and replicate at a temperature of 44.5 °C. They were then stored again at a very low temperature (2-4°C) for a period of two to three weeks and the process is repeated again.

Introduction of the *E-Coli* to the broth media is performed in the same way except that after the smearing on the surface of the cultured agar plate using the sterilized rod, the rod is dipped into the broth solution and mixed well with the solution. The broth tubes are then incubated for 24 hours, and the *E. coli* populations can be visually identified due to the change in coloration of the media (from deep purple to yellow).

3.5.2 Buffer Solution Stability

Under normal room temperatures (23 to 25 °C), the survival of the bacteria follows the three phases mentioned previously, and the bacteria population eventually dies out as soon as the nutrients in the media are exhausted. The aim of this experiment was to construct the survival curve of the *E. coli* strain: the concentration of the bacteria versus time. This experiment was done according to the following procedure (Hegazy (2001) and Badawy (2005)):

- One of the bacteria cultures in the broth solution is taken from the low temperature fridge in which they are kept inactive, and placed in the incubator at 44.5 ˚C for about an hour.
- The tube is then taken out of the incubator and placed in a shaker to rotate at a speed of 600 rpm for 10 to 15 minutes.
- A sample of 1 ml of the solution was taken using a sterilized micropipette, and inputted in a distilled water of volume 1L dissolved with approximately a third of HACH BOD Nutrient buffer pillow.
- The mixture was then shaken very well.
- Assuming that the original culture plate has colonies in the count of billions (n x 10^9), further dilution was done in the same way by taking 5 ml of the buffer prepared in the previous step and inputting it in another solution of 1 L distilled water mixed with a third of a HACH BOD Nutrients pillow.
- Using a sterilized micropipette, 3 samples each of 1 ml volume were drawn from the final buffer prepared and were dissolved in 3 volumetric flasks each of which contains 100 ml distilled water.
- These solutions were then filtered and cultured on m-fecal media plates that were previously prepared according to the instructions of the manufacturer. These three plates were then marked as the concentration of the bacterial colonies at time $t = 0$
- These two previous steps were repeated for t= 0.25 hr., 1 hr, 1 day, 2 days, 2.5 days, 7 days, 8 days, and 11 days. The curve was then constructed as the relative concentration (concentration at time t/original concentration) versus time.

The concentration of the buffers as well as the original cultured plate can be determined from the final plate count after a series of dilutions as shown in the following example illustrated in **Figure 3-3**:

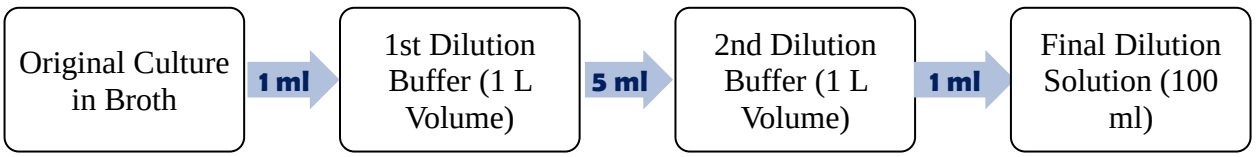

Figure 3-3 - Bacteria Count Series of Dilutions

If, for example, after incubation, the plates cultured from the last dilution read a concentration of 50 colonies per 100 ml, the previous concentrations can be determined as follows:

- Concentration of 2nd buffer = 50 × 100 ml/1 ml = 5,000 colonies per 100 ml
- Concentration of 1st buffer = 5,000 × 1000 ml/5 ml = 5,000 × 200 = 1,000,000 colonies per 100 ml
- Concentration of the original broth culture = 1,000,000 × 1000 ml/1 ml = 1,000,000,000 colonies per 100 ml → 1×10^9 Colonies per 100 ml

These are the typical calculations done for counting of the bacteria whether in a buffer solution or to determine the concentration of the original culture in broth.

3.6 Bacteria Survival Experiments

The bacteria survival experiments are experiments performed using the same procedures but done at different conditions. The main independent variables in these experiments are the organic content of the soil, the concentration of the applied buffer solution in the experiment, and the temperature to which the soil columns are exposed. **Before the** start of the experiment, on the same day, the following materials are needed:

- Soil that is dry and sterile with identified organic content and sieved to 2 mm in particle size using an ASTM E11 sieve.
- Seven 5 cm PVC columns: clean, sterile, and dry (these columns were previously prepared as explained in **Section 3.3.**
- Buffer solution to be prepared prior to the experiment the same way as described in the buffer stability procedures to the desired target concentration.
- Ten 100 ml graduated cylinders or volumetric flasks that are clean and sterile.
- At least 12 ready-made m-fecal agar media plates.
- 5 clean and sterile high density 250 ml polyethylene bottles.
- Sterile pipettes and micropipettes
- 10 Clean and sterile filter units set up with sterile disposable membranes with pore size 0.45 μm and 47 mm in diameter.
- Pumps to be used for filtration.

Table 3-2 summarizes how these experiments are divided.

Table 3-2 - Bacteria Survival Experiments Categorized

Low Organic Content Soil Low Concentration Buffer Low Temperature	Medium Organic Content Soil Low Concentration Buffer Low Temperature	High Organic Content Soil Low Concentration Buffer Low Temperature
Low Organic Content Soil Medium Concentration Buffer Low Temperature	Medium Organic Content Soil Medium Concentration Buffer Low Temperature	High Organic Content Soil Medium Concentration Buffer Low Temperature
Low Organic Content Soil High Concentration Buffer Low Temperature	Medium Organic Content Soil High Concentration Buffer Low Temperature	High Organic Content Soil High Concentration Buffer Low Temperature
Low Organic Content Soil Low Concentration Buffer High Temperature	Medium Organic Content Soil Low Concentration Buffer High Temperature	High Organic Content Soil Low Concentration Buffer High Temperature
Low Organic Content Soil Medium Concentration Buffer High Temperature	Medium Organic Content Soil Medium Concentration Buffer High Temperature	High Organic Content Soil Medium Concentration Buffer High Temperature
Low Organic Content Soil High Concentration Buffer High Temperature	Medium Organic Content Soil High Concentration Buffer High Temperature	High Organic Content Soil High Concentration Buffer High Temperature

After its preparation, the bacterial buffer solution was kept in a covered 3 liter PVC bottle, and was sealed well until use in the experiment. For use, the buffer solution was introduced carefully to the surface of the soil column in a swirling fashion. The soil used in these experiments is the sandy soil (AUC soil) described previously, and the three buffer concentrations targeted for preparation were 500 CFU/100 ml, 5000 CFU/100 ml, and 50,000 CFU/100 ml. Each of these experiments is typically done in a time frame of at least one week.

Before the start of the experiment, on the same day, the following materials are needed:

- Soil that is dry and sterile with identified organic content and sieved to 2 mm in particle size using an ASTM E11 sieve.
- Seven 5 cm PVC columns: clean, sterile, and dry (these columns were previously prepared as explained in **Section 3.3.**
- Buffer solution to be prepared prior to the experiment the same way as described in the buffer stability procedures to the desired target concentration.

- Ten 100 ml graduated cylinders or volumetric flasks that are clean and sterile.
- At least 12 ready-made m-fecal agar media plates.
- 5 clean and sterile high density 250 ml polyethylene bottles.
- Sterile pipettes and micropipettes
- 10 Clean and sterile filter units set up with sterile disposable membranes with pore size 0.45 μm and 47 mm in diameter.
- Pumps to be used for filtration.

In performing each of the experiments stated in **Table 3-2**, the following procedures are followed:

- The set of seven soil columns is carefully packed one day before the start of the experiment to save time, and to avoid contamination of soil after it has been packed, each soil columns was sealed using aluminum foils.
- One pore volume (PV = Volume of Sand Column × Porosity) is applied slowly and carefully to each of the columns to avoid the causation of surface ponds on top of the soil columns. Funnels were placed underneath each of the columns to note and measure any dripping. (PV calculated for Regular Soil = 67.2 ml, for Soil with 0.3% Organic Matter = 62.3 ml, and for soil with 0.5% Organic Matter = 61.1 ml)
- The columns were then placed on trays, and the trays placed on the roof.
- One column was removed for analysis at the following times: after 30 minutes, after 8-10 hours, after 24 hours, after 48 hours, after 72 hours, after 5 days, and after 7 days.
- The concentration of the bacteria was studied in each of these columns with respect to the depth: *i.e.* sampling of the soil for testing from the column was done for various depths of the column (1 cm, 2 cm, 3 cm, 4 cm, and 5 cm samples). Accordingly, the study of each column was conducted in the following steps (for each of the specified times):
    1. The column is weighed and compared to the weight of the packed soil column before adding the buffer solution in order to obtain an estimate of moisture content at the give time.
    2. First, the clip at the bottom of the column was carefully removed, and using a plunger, the soil was pushed upwards until 1 cm is exposed. This 1 cm was then cut using a sterile knife. This was done for each of the five centimeters (the total height of the column).
    3. Each of these sliced soils was then mixed, and a sample of 10 grams was weighed out of each of them and placed in each of the 5 polyethelene bottles.
    4. A volume of (100 – 10 gm/ρ_b) of distilled water is then added to each of these bottles containing 10 gm of soil samples each.

5. The five bottles are then placed in the shaker and allowed to rotate for 20 minutes at 400 rpm (revolutions per minute).
6. 2 samples, each of 0.1 to 1 ml, are drawn from a bottle and inputted into 2 100 ml volumetric flasks or graduated cylinders filled with distilled water. This was repeated for each of the five bottles until all 10 flasks/cylinders are ready for filtration.
7. Finally, filtration was done using the pump and the filter units and membranes, and duplicates of culture plates were made for each sample. Note that on the first reading (at 30 minutes), 2 plates of blanks have to be prepared as well as the samples (making them 12 plates in total). The blanks are done according to the series of dilutions that were previously illustrated in this section.

4 Results and Discussion

4.1 Soil Properties

As indicated in the previous chapter (Materials and Methods), the soil used in all experiments was a sandy, desert soil. The following properties were determined experimentally: 1) bulk density of the soil; 2) particle density of the soil; 3) porosity, calculated from the previous two; and 4) total organic content (TOC) in the soil.

Table 4-1 Soil Properties

Property	Value Obtained	Units
1. Bulk Density of soil	1.615 ± 0.004	g/L
2. Particle Density of soil	2.662 ± 0.003	g/L
3. Porosity of soil	39.30 ± 0.13	%
4. pH of soil	4.00 ± 0.15	
5. TOC	0.035 ± 0.001	%
6. Bulk Density of soil with 0.3 % organic matter	1.613 ± 0.002	g/L
7. Particle Density of soil with 0.3 % organic matter	2.545 ± 0.004	g/L
8. Porosity of soil with 0.3 % organic matter	36.6 ± 0.162	%
9. Bulk Density of soil with 0.5 % organic matter	1.613 ± 0.008	g/L
10. Particle Density of soil with 0.5 % organic matter	2.511 ± 0.010	g/L
11. Porosity of soil with 0.5 % organic matter	35.80 ± 0.25	%

Based on the above data, the sandy soil used in these experiments was highly porous (39.3% pores of total volume) and with relatively low organic content. The porosity obtained is consistent with the range of porosities indicated for sandy soils between 0.25 and 0.5 (SPE International).

A sieve analysis for the test soil is shown in **Table 4-2, Figure 4-1**, and **Figure 4-2**. The data indicate that the grain size of the sandy soil obtained for use in this study varies between 0.075 mm to 4.75 mm grains. However, the 0.075 mm grain size represents only about one percent of the sample. The majority of the grain sizes of the soil range in size between 0.15 mm to 0.425 mm (retained = 35.7%).

Table 4-2 Sieve Analysis Results

US Sieve Number	Grain Sizes (mm)	Cumulative Weight Retained (g)	Weight Retained (g)	Cumulative Weight Passing (g)	% Retained	% Passing	% Cumulative Passing
4	4.75	17	17	483	3.4	96.6	96.6
8	2.36	32.5	15.5	467.5	3.1	96.9	93.5
10	2	37	4.5	463	0.9	99.1	92.6
20	0.85	98	61	402	12.2	87.8	80.4
30	0.6	169.5	71.5	330.5	14.3	85.7	66.1
40	0.425	259	89.5	241	17.9	82.1	48.2
100	0.15	437.5	178.5	62.5	35.7	64.3	12.5
200	0.075	494	56.5	6	11.3	88.7	1.2

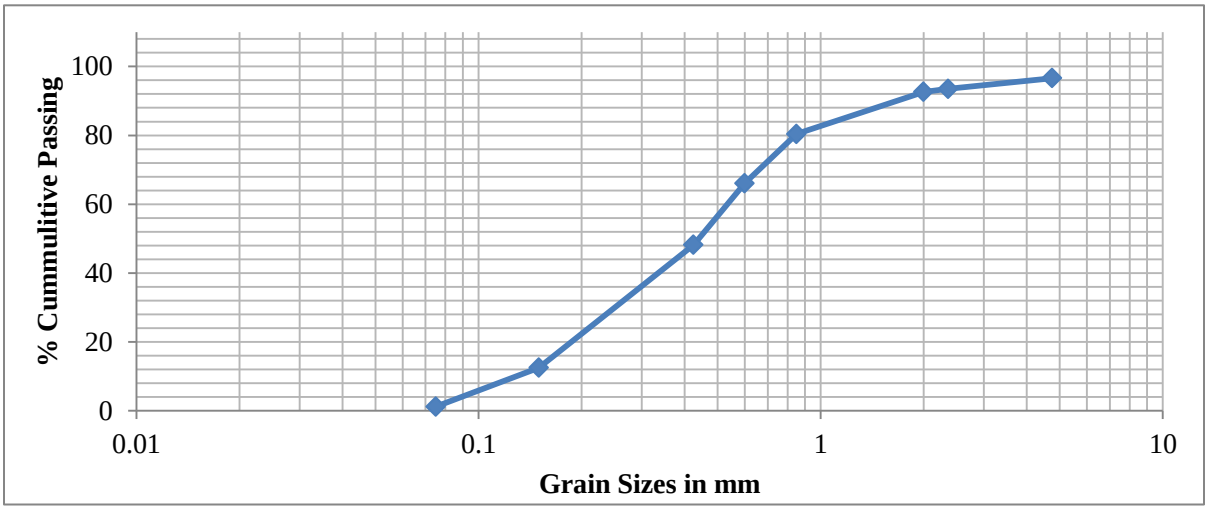

Figure 4-1 – Cumulative Passing from Soil vs. Grain Size

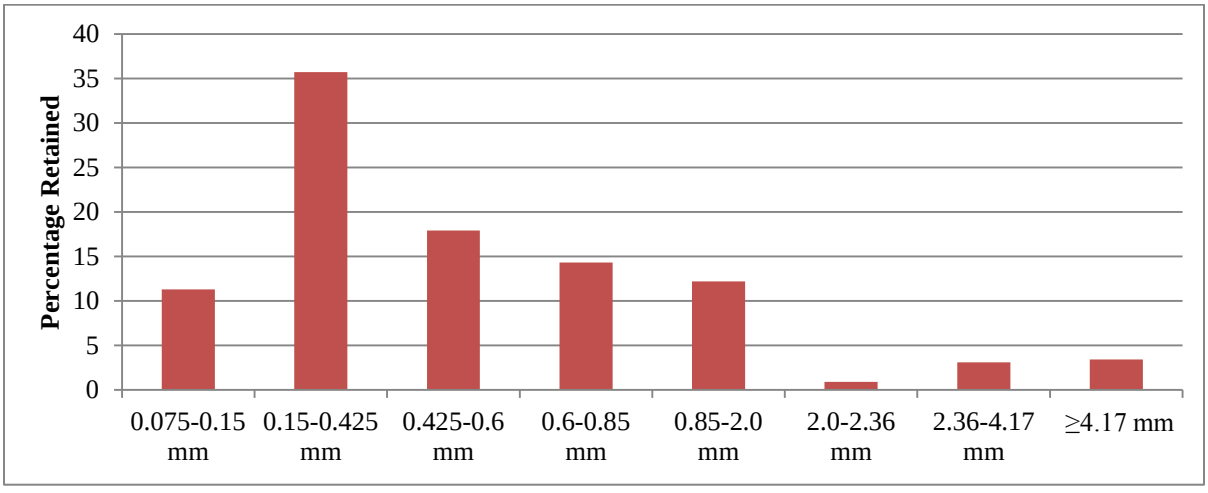

Figure 4-2 - Percentage Retained vs. Soil Particle Size Range

IR Analysis

The purpose of the IR tests was to identify organic surface functional groups on the test soil, and to determine whether autoclaving the soil alters the surface character of the test soil. Accordingly, the IR tests were performed for the dry soil 'as is' and an autoclaved sample. Both samples exhibited essentially identical spectra, suggesting that autoclaving the soil as done in this work did not alter its structure. The likely surface groups existing on the soil as interpreted according to those peaks are given in the following table. The IR analysis also indicated consistency with the results obtained from the organic content analysis in that the amount of organic content is low.

Table 4-3 IR Analysis Results

Frequency (cm^{-1})	Corresponding Bond
3020.5 (br) Stretch	O-H for Alcohols or Phenols
1080 (s) Stretch	C-O for Alcohols, Ethers, Carboxylic acids, or Esters
779.2 (s) bend	C-H for Alkenes

4.2 Buffer Stability Experiment Results

The purpose of the buffer stability experiment was to determine the approximate time at which the bacterial population reaches its maximum, and its approximate survival time. This experiment was therefore conducted in the laboratory at room temperature (23 °C) without any exposure to sunlight or ambient atmosphere. The initial concentration was measured and recorded as C_o, and samples were collected throughout a time frame of 7 days as shown in Table 4-3: after 15 minutes (0.25 hours), an hour, 6 hours, 24 hours, 48 hours (2 days), 60 hours (2.5 days), and 7 days. A, B, and C represent the plate counts of the diluted buffer solution of the indicator bacteria E-coli sample collected at time t. The calculated relative concentration C/C_o represents the change in the concentration of the buffer C at a time t (in days) with respect to the original concentration C_o taken at the start of the experiment (t= 0).

The results, as shown in Table 4-4, indicate that the survival pattern for the bacterial population involves a small growth period followed by a die off (or decay) period. The results in Table 4-4 as well as in Figure 4-3 show that, allowing for standard deviation, the bacterial population is relatively stable during the initial 24 hours, after which it starts decaying exponentially until the entire population dies off completely at day 7. These results are consistent with the general survival trend of the bacteria under normal conditions as well as previous studies with similar strains of *E-coli* (Badawy, 2005).

Table 4-4 Data from Buffer Stability Analysis

Hour	A	B	C	Average Count	Standard Deviation	C/C_o
0	26	27	28	27	1.00	1
0.25	24	27	30	27	3.00	1
1	25	28	35	29.33	5.13	1.086
6	30	35	37	34	3.61	1.259
24	N/A	22	31	26.5	6.36	0.981
48	15	19	23	19	4.00	0.704
60	5	6	7	6	1.00	0.222
168	0	0	0	0	0.00	0

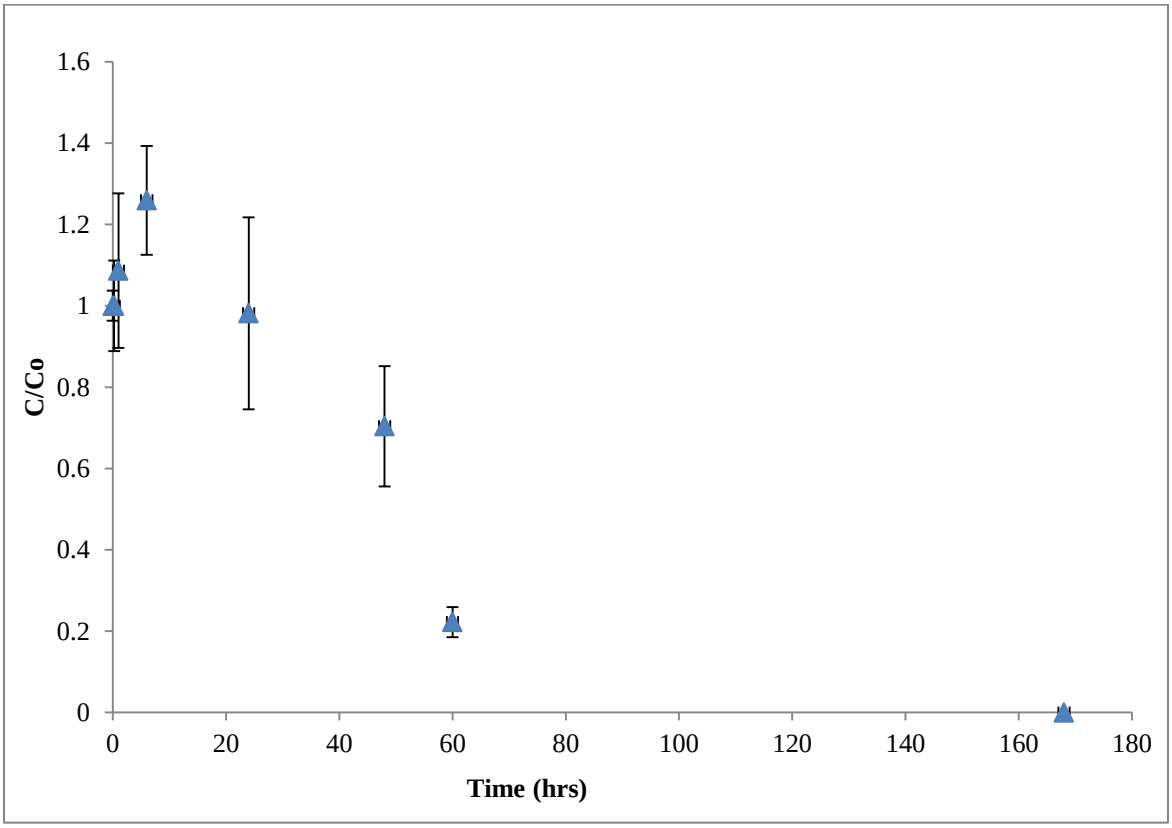

Figure 4-3 - Relative Bacterial Concentration vs. Time from Buffer Stability Analysis

4.3 Bacteria Survival Experiments

The bacteria survival tests were essentially static column experiments that were conducted with the same (AUC) soil but with changes in the organic content through the application of horse manure as explained in Chapter 3. The soil columns were exposed to outdoor environmental conditions (sunlight, temperature changes, rain…etc.), and the experiments were repeated for each soil organic fraction both during summer and winter seasons. The first soil column was analyzed after 30 minutes (0.5 hours) from applying the bacterial buffer solution, and samples were analyzed from depths 0.5 cm, 1.5 cm, 2.5 cm, 3.5 cm, and 4.5 cm respectively of the entire column. Similarly, samples were analyzed from each of the remaining 6 columns after 8-12 hours, 24 hours, 48 hours, 72 hours, 120 hours, and 168 hours, respectively. On several occasions, it was noticed that the counts for the bacteria were much too high even for a sample of as high a dilution as 1:200. Accordingly, the data collected in such case represent the approximate count for each of the samples collected instead of the exact number of the bacterial colonies in the particular sample, and this is illustrated in **Table 4-5**.

Table 4-5 – Raw Data for Bacteria Survival Experiment with "As Is" AUC Soil (0.035% Organic Content) with High Concentration Buffer and High Temperature

		Plate Count	0.5 cm	1.5 cm	2 cm	3 cm	4 cm
Plate Factor: 200	**Col. 1 - 0.5 hrs.**	***P1***	48	30	42	40	42
		P2	46	36	60	40	54
		Average	47	33	51	40	48
	Col. 2 - 9 hrs.	***P1***	48	30	72	42	96
		P2	36	30	60	42	84
		Average	42	30	66	42	90
	Col. 3 - 24 hrs.	***P1***	168	190	276	392	1088
		P2	160	210	352	412	1098
		Average	164	200	314	402	1093
	Col. 4 - 48 hrs.	***P1***	160	200	320	480	1300
		P2	140	280	350	500	1200
		Average	150	240	335	490	1250
Plate Factor: 100	**Col. 5 - 72 hrs.**	***P1***	6	1344	2160	2400	2720
		P2	4	1320	2150	2330	2700
		Average	5	1332	2155	2365	2710
	Col. 6 - 5 days	***P1***	N/D	20	30	32	35
		P2	N/D	18	24	34	37
		Average	N/D	19	27	33	36
Plate Factor: 40	**Col. 7 - 7 days**	***P1***	N/D	N/D	N/D	1	2
		P2	N/D	N/D	N/D	1	2
		Average	N/D	N/D	N/D	1	2

Target Buffer Solution Concentration: 50,000 CFU/100 ml – Average Concentration = 45000 CFU/100ml (C1 Recovered = 44000 CFU/100 ml ; C2 Recovered = 46000 CFU/100 ml)
Soil Organic Content: 0.035%
Pore Volume of Buffer used = 67.2 ml

Table 4-6 Bacteria Concentration Profile at High Temperature with Various Initial Concentrations for "As Is" AUC soil (0.035% Organic Content)

High Temperature Season (35 – 30 °C)								
Depth	Average Initial conc.	Time (Hour)						
(cm)	**CFU/100 ml**	**0.5**	**9**	**24**	**48**	**72**	**120**	**168**
0.5	45000	9400	8400	32800	30000	500	0	0
	±1414	*±283*	*±1697*	*±1131*	*±2828*	*±141*	*0*	*0*
	6850	*0*	*141*	*0*	*0*	*71*	*20*	*31*
	±71	*0*	*±100*	*0*	*0*	*±50*	*±10*	*0*
	540	45	10	40	5	0	0	0
	±28	*±7*	*0*	*±14*	*0*	*0*	*0*	*0*
1.5	45000	6600	6000	40000	48000	133200	1900	0
	±1414	*±848*	*0*	*±2828*	*±11,313*	*±1697*	*±141*	*0*
	6850	700	1100	4500	466.6667	450	120	0
	±71	*0*	*±100*	*±100*	*±115*	*±50*	*±20*	*0*
	540	25	15	40	15	0	0	0
	±28	*±7*	*±7*	*0*	*±7*	*0*	*0*	*0*
2.5	45000	10200	13200	62800	67000	215500	2700	0
	±1414	*±2545*	*±1697*	*±10748*	*±4243*	*±707*	*±424*	*0*
	6850	1000	1400	5733	1600	417	0	0
	±71	*0*	*±100*	*±306*	*±200*	*±58*	*0*	*0*
	540	20	45	85	0	0	0	0
	±28	*±14*	*±7*	*±21*	*0*	*0*	*0*	*0*
3.5	45000	8000	8400	80400	98000	236500	3300	40
	±1414	*0*	*0*	*±2828*	*±2828*	*±4950*	*±141*	*0*
	6850	2200	2600	13400	2200	600	0	0
	±71	*±200*	*±173*	*±200*	*±200*	*±50*	*0*	*0*
	540	400	30	155	5	0	0	0
	±28	*±7*	*0*	*±7*	*0*	*0*	*0*	*0*
4.5	45000	9600	18000	218600	250000	271000	3600	80
	±1414	*±1697*	*±1697*	*±1414*	*±14142*	*±1414*	*±141*	*0*
	6850	3100	3400	23200	4400	2650	600	460
	±71	*±100*	*±173*	*±200*	*±200*	*±50*	*±70*	*±31*
	540	50	35	130	0	0	0	0
	±28	*±14*	*±7*	*±14*	*0*	*0*	*0*	*0*

Table 4-7 Bacteria Concentration Profile at Low Temperature with Various Initial Concentrations for "As Is" AUC soil (0.035% Organic Content)

Low Temperature Season (22 – 12 °C)								
Depth	Average Initial conc.	**Time (Hour)**						
(cm)	**CFU/100 ml**	**0.5**	**9**	**24**	**48**	**72**	**120**	**168**
0.5	36250	9800	8400	5550	50	1075	780	0
	±1768	*±283*	*±566*	*±495*	*±7*	*±141*	*±28*	*0*
	3600	610	280	360	10000	250	6500	2500
	±141	*±14*	*±28*	*±57*	*±707*	*±35*	*±141*	*±141*
	450	50	33	140	588	44	32	14
	0	*±10*	*±12*	*±20*	*±17*	*±6*	*±6*	*±1*
1.5	36250	10100	6200	0	0	1375	660	0
	±1768	*±424*	*±566*	*0*	*0*	*±212*	*±57*	*0*
	3600	610	370	390	3350	40000	79200	110
	±141	*±71*	*±14*	*±42*	*±71*	*±1768*	*±1131*	*±42*
	450	110	80	20	50	54	34	22
	0	*±20*	*±28*	*±12*	*±14*	*±3*	*±8*	*±1*
2.5	36250	7800	3900	1300	0	525	280	0
	±1768	*±283*	*±707*	*±283*	*0*	*±106*	*±56*	*0*
	3600	660	160	430	5460	19700	52000	80
	±141	*±28*	*±57*	*±42*	*±651*	*±424*	*±2828*	*±28*
	450	160	140	40	772	696	36	24
	0	*±10*	*±20*	*0*	*±40*	*±23*	*±6*	*±1*
3.5	36250	5200	2600	600	50	475	60	0
	±1768	*±283*	*±566*	*±141*	*±21*	*±35*	*±28*	*0*
	3600	800	260	480	3300	21750	81000	180
	±141	*±28*	*±28*	*±28*	*±141*	*±1060*	*±1414*	*±28*
	450	57	100	87	72	1100	46	24
	0	*±6*	*0*	*±12*	*±11*	*±28*	*±8*	*0*
4.5	36250	3100	1300	400	0	100	0	0
	±1768	*±141*	*±141*	*±141*	*0*	*0*	*0*	*0*
	3600	410	70	100	3720	34900	14200	280
	±141	*±14*	*±42*	*±28*	*±113*	*±141*	*±283*	*±57*
	450	23	47	80	164	76	16	0
	0	*±15*	*±12*	*±20*	*±6*	*±6*	*±6*	*0*

The sampled wet weight of soil from each column was 10 g respectively and as previously mentioned, the dilution factor varied between the ratio (200:1) to (10:1) depending on the initial concentration of the used buffer solution in the soil and the recovered concentration from the previous sampling.

To estimate the zero-order growth rate coefficient and the decay rate for the bacteria in the soil, the concentrations at each depth (in CFU/100 ml) were first calculated from the plate counts, such as the values presented in **Table 4-5**. Calculated concentrations are presented in **Table 4-6** and **Table 4-7** for the 'as is' soil experiments conducted during the summer and the winter, while the soil columns water content is given in **Tables 4-8** and **4-9** respectively. Accordingly, the total cells in a soil column at the designated time can then be estimated according to the equation:

$$\text{Total Cells in Column} = \sum \text{Concentrations for all depths} \times \left[\frac{(\text{Mass Soil} + \text{Mass Water})}{50}\right]$$

Table 4-8 Averaged Estimation of Soil Water Content during the High Temperature Season

Col.	Dry	Final	Water (g)
1	410	485	75
2	410	425	15
3	410	415	5
4	410	412	2
5	410	410	0
6	410	410	0
7	410	410	0

Table 4-9 Averaged Estimation of Soil Water Content during Low Temperature Season

Col.	Dry	Final	Water (g)
1	410	485	75
2	410	440	30
3	410	430	20
4	410	420	10
5	410	420	10
6	410	416	6
7	410	410	0

The number of bacterial cells until they reach the maximum value were then plotted against the equivalent time frame. Once the bacterial cells have reached the maximum value, the number of cells starts decreasing indicating decay. During the time frame from the maximum value until the minimum is reached, the values were plotted versus time to obtain the decay rate coefficient. An example of this procedure is shown in **Figure 4-4** and **Figure 4-5** obtained from the experiment conducted with the "as is" AUC soil with high initial buffer concentration (45,000 CFU/100 ml) during the summer at high temperature. Whereas **Figure 4-4** illustrates that the growth phase is nearly linear, representing zero-order rate, **Figure 4-5** is more indicative of first-order exponential decay for the bacteria under the same conditions after their maximum growth is reached.

Figure 4-4 and **Figure 4-5**, however, represent only one set of the bacterial survival experiments. Similar plots were established, which will be shown later on, in order to estimate the growth and decay rates of the bacterial cells in the soil for other conditions. The two main reasons why zero-order growth model was used to represent the growth of the bacterial cells with time and estimate the growth coefficient are: 1) the data for the growth phase of the bacteria in the soil fit better into a linear trend more than first-order fit and this was determined from the R^2 values; and 2) to follow a previous study related to the same field and compare the two cases (Badawy, 2005).

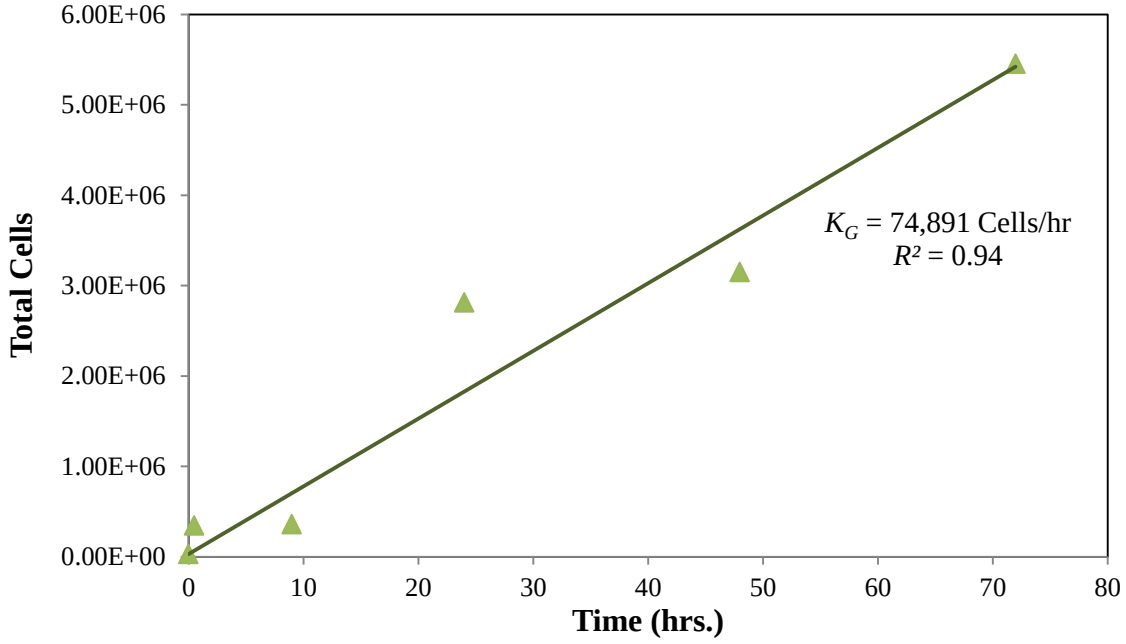

Figure 4-4 Estimation of Zero Order Growth Coefficient in Soil "as is" at High Temperature

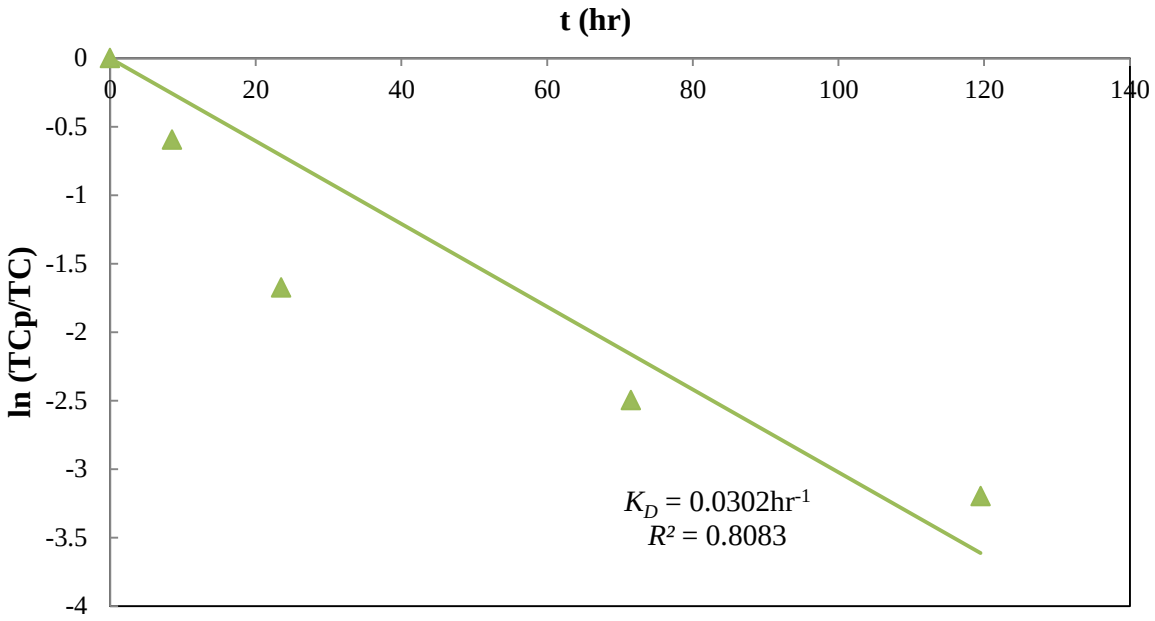

Figure 4-5 Estimation of First-Order Exponential Decay Coefficient in Soil "as is" at High Temperature

The variation in the bacteria survival kinetics was found to not only depend on the type of the soil (specifically in terms of its organic content) but also on the seasonal temperature as well as the initial concentration of the bacterial buffer solution applied to the soil. **Tables 4-10**, **4-11**, and **4-12** summarize the observations of *E-coli* growth and decay for the complete array of experiments, and the following sections present the detailed data, analysis, and discussion.

Table 4-10 - Summary of Changes Observed in Bacteria Survival with the Use of High Concentration Buffer Solution (Average 50,000 CFU/100 ml)

.	0.035 % O.C. Soil	0.3 % O.C. Soil	0.5 % O.C. Soil
30 – 35 °C	Extent of growth is relatively low (Relative Cells ≈ 180) and the growth rate is low (maximum cells after 72 hours), followed by rapid decay.	Extent of growth is higher (Relative Cells ≈ 432) while the growth rate is high (24 hours only), followed by a rapid decay.	Extent of growth is highest (Relative Cells ≈ 1011) along with a high growth rate (extends to 24 hours only), followed by slow decay.
12 – 22 °C	No bacterial growth was noticed during this set of experiments, where the concentration measured after 30 minutes was almost the same as the initial concentration (Co = 35000 CFU/100 ml, and C = 36000) but the decay rate observed was low (extends to 7 days).	Extent of growth is low (Relative Cells ≈ 48) with high growth rate (extends to 24 hours only), followed by rapid decay.	Extent of growth is very low – almost unnoticeable (Relative Cells ≈ 12) and is reached only after 9 hours, which is then followed by slow decay.

Table 4-11 - Summary of Changes Observed with Bacteria Survival with the Use of Medium Concentration Buffer Solution (Average 5,000 CFU/100 ml)

.	0.035 % O.C. Soil	0.3 % O.C. Soil	0.5 % O.C. Soil
30 – 35 °C	Extent of growth is low (Relative Cells ≈ 61) but the growth rate is high (extends to 24 hours only), and is followed by slow decay.	Extent of growth is high (Relative Cells ≈ 521) and the growth rate is also high (24 hours only), followed by a rapid decay.	Extent of growth is high (Relative Cells ≈ 211) along with a high growth rate (extends to 24 hours only), followed by slow decay.
12 – 22 °C	Extent of growth is more than ten times higher than in the summer (Relative Cells ≈ 626) but the growth rate is low (extends to 120 hours) followed by rapid decay.	Extent of growth is much higher than that in the summer (Relative Cells ≈ 2851) with high growth rate (extends to 24 hours only), followed by rapid decay.	Extent of growth is extremely high (Relative Cells ≈ 4928) accompanied by low growth rate (extends to 120 hours), followed by rapid decay.

Table 4-12 - Summary of Changes Observed with Bacteria Survival with the Use of Low Concentration Buffer Solution (Average 500 CFU/100 ml)

.	0.035 % O.C. Soil	0.3 % O.C. Soil	0.5 % O.C. Soil
30 – 35 °C	Almost no growth of the bacteria was noticed at all – rather, a stagnation period (extends to 24 hours only), followed by rapid decay.	Extent of growth is high (Relative Cells ≈ 664) along with high growth rate (extends to 24 hours only), followed by a rapid decay.	Extent of growth is highest (Relative Cells ≈ 976) accompanied by high growth rate (extends to 24 hours only), followed by rapid decay.
12 – 22 °C	Extent of growth is very low (Relative Cells ≈ 39) with low growth rate (extends to 72 hours), followed by relatively slow decay.	Extent of growth is high – though lower than in the summer (Relative Cells ≈ 212) with high growth rate (extends to 24 hours only), followed by slow decay.	Extent of growth is high – though lower than in the summer (Relative Cells ≈ 792) with low growth rate (extends to 72 hours), followed by rapid decay.

4.3.1 Seasonal Experiments Using Soil of Low Organic Content

The first set of the bacteria survival experiments were done on the AUC soil "as is", without adding any horse manure to increase its organic content; *i.e.* soil with only 0.035% organic content. The recorded temperature during experimentation ranged between 30°C and 35°C in the summer and between 12°C to 22°C in the winter. The target or theoretical initial concentrations of the bacterial solutions applied were 50,000 CFU/100 ml, 5000 CFU/100 ml, and 500 CFU/100 ml, respectively. During the preparation of

these solutions, the exact concentration could not always be obtained. For the 50,000 CFU/100 ml case, for instance, sometimes the concentration prepared was as low as 35,000 CFU/100 ml or as high as 75,000 CFU/100 ml instead of the targeted concentration. The E-coli showed a similar pattern of survival as in the buffer stability analysis in its growth and then exponential decay. However for the sake of comparison, the theoretical values will be used to categorize the differences in the bacteria survival pattern with time and depth.

Figure 4-6 and **Figure 4-7** illustrate the trend of bacteria survival for different initial buffer concentrations of *E-coli* to the soil columns recorded throughout a time frame of 168 hours conducted during the high temperature season and the low temperature seasons, respectively. At such a low concentration of organic matter in the soil, the major contributing factors to the change in bacterial survival pattern appear to be the temperature and exposure to sunlight, and the initial concentration of the bacterial solution applied to the soil.

In **Figure 4-6**, the results show relatively high growth rates of the *E-coli* bacteria in the soil during the first 24 - 72 hours followed by a rapid decay rate except for the case of a medium concentration buffer (4500 CFU /100 ml) where a slower decay rate was observed during the summer. It was also noticed that the extent of increase in the relative total cells increased with increasing initial concentrations. In case of the 45,000 CFU/100 ml application the relative bacterial cells reached a value of 180 after 72 hours, a value of 61 after only 24 hours in case of the 7,500 CFU/100 ml buffer, and about 12 for the low concentration buffer.

Conversely, the winter season results presented in **Figure 4-7** indicate that the growth of the *E-coli* bacteria in the soil may occur over a much longer period than in the summer. As long as 3 – 5 days was required to reach the maximum, after which this growth is followed by a slow decay rate except for the case of a medium concentration buffer (7,500 CFU /100 ml) where a more rapid decay rate was noticed during the winter (in contrast to what was observed in the summer). The extent of increase for the lower and medium range buffer concentrations (500 and 3,600 CFU/100ml) was greater at lower temperatures than at higher temperatures. Interestingly, and very different from the high temperature case, the extent of relative bacteria growth was lowest for the high concentration buffer. The reason for this is difficult to know, especially given the unpredictability of analyzing living organisms in a natural system. In case of the 3600 CFU/100 ml application the relative bacterial cells reached a value of 625 after 5 days, while it reaches a value of 39 also after 5 days in case of the application of 500 CFU/100 ml buffer.

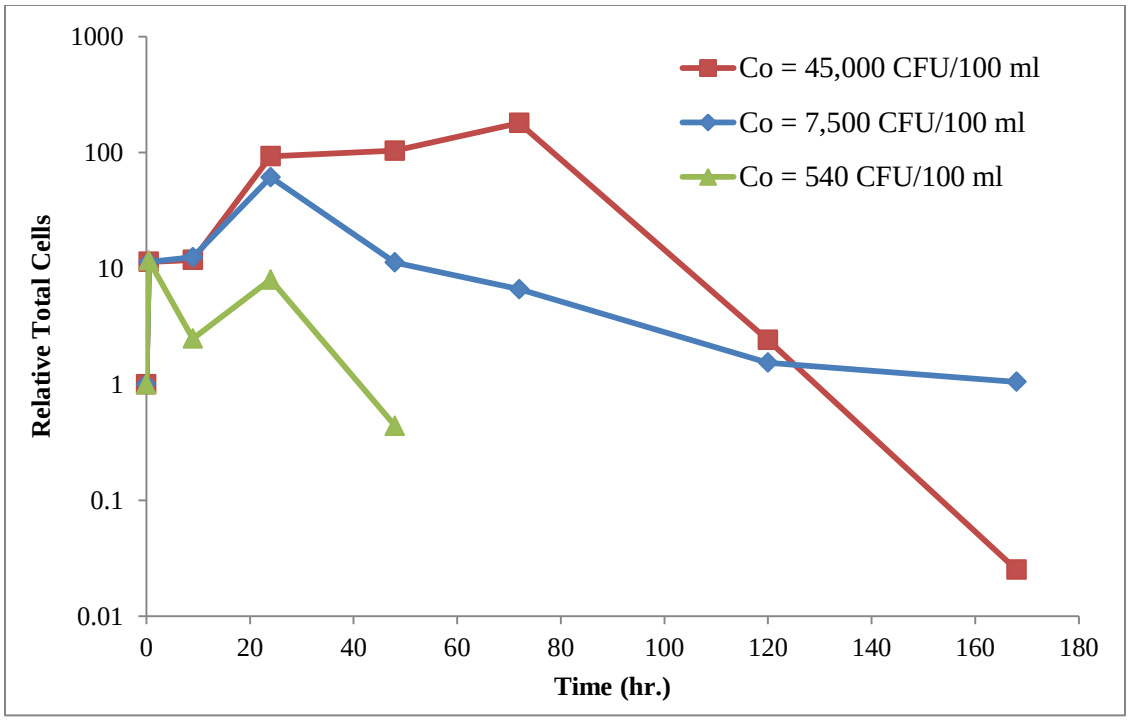

Figure 4-6 - Relative Total Cells vs. Time in As Is Soil at High Temperature for Various Initial *E-coli* concentrations

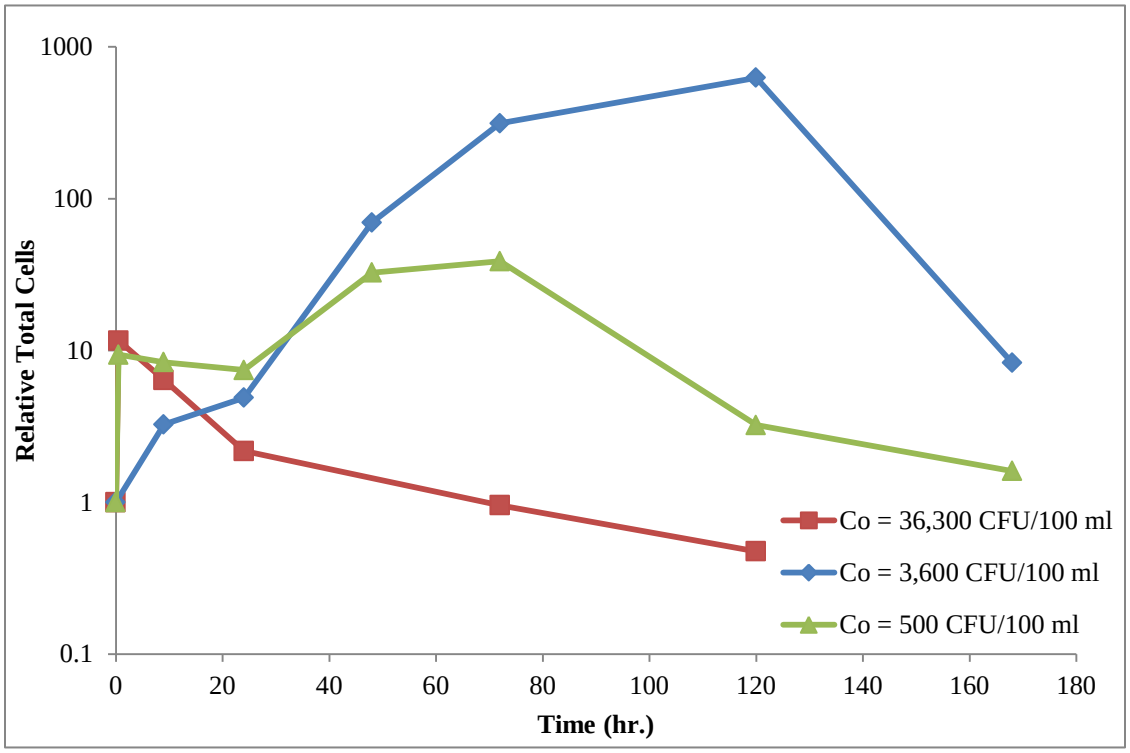

Figure 4-7 - Relative Total Cells vs. Time in As Is Soil at Low Temperature for Various Initial *E-coli* concentrations

4.3.2 Seasonal Experiments Using Soil of Medium (0.3%) Organic Content

The following set of the bacteria survival experiments were done on the same AUC soil after adding horse manure to it in order to increase its organic content from 0.035% to 0.3%. As was the case in the first set, the recorded temperature also ranged between 30°C and 35°C in the summer and between 22°C to 12°C in the winter. Also, the target or the theoretical initial concentrations of the bacterial solutions introduced to the soil columns were 50,000 CFU/100 ml, 5000 CFU/100 ml, and 500 CFU/100 ml respectively, and as it was previously noted, it was not always possible to obtain the exact concentration as the target values. The average concentrations of the prepared solutions ranged between 36,000 CFU/100 ml and 57,500 CFU/100 ml instead of the value 50,000 CFU/100 ml, and between 4000 CFU/100 ml and 4800 CFU/100 ml instead of an exact value of 5000 CFU/100 ml. On the other hand, the average concentration of the prepared low buffer concentration was exactly measured as targeted (500 CFU/100 ml).

Figure 4-8 and **Figure 4-9** represent the survival trend of the E-coli using the relative bacterial cells retrieved throughout the experimentation period for high and low temperatures, respectively. Conversely to the previous set, however, the much more noticeable general trend indicated by **Figure 4-8** is the high growth rate of the bacteria followed by a high decay rate. This is also shown in the values of total concentrations, the relative concentrations, and the total cells of each column recorded in Error! eference source not found.. Although it was expected for the increase of organic content to increase the survival of the bacteria, the opposite scenario seems to have occurred: the growth of the bacteria only lasts for 24 hours duration where it reaches its maximum and then starts decaying. However, in this case, due to the presence of the organic content, the extent of growth of the bacterial population in the soil greatly increased.

As previously, mentioned in Chapter 3, with the presence of organic content, the heat and sunlight work as a catalyst that speeds up both the growth rate and the decay rate as it is shown in the values of the total cells of each column in **Table 4-13** Table **4-14** and in the diagrams in **Figure 4-8** and **Figure 4-9** at both high and low temperatures. Moreover, the data presented in **Table 4-13** and **Table 4-14** show that at 24 hours, the bacterial population reaches its maximum growth not only in the value of its concentration, but also throughout the entire column at every depth; *i.e.* the increase happens in each of the individual layers of the soil.

Figure 4-8 indicates that the bacterial increase, when the concentration of the buffer applied is as low as 430 CFU/100 ml, is highest during the summer where there is extensive heat (30 – 35 °C) and abundance of sunlight, for which the value of the relative cells is 664 after 24 hours. The relative cells indicating the

extent of growth of *E-coli*, as shown in the diagram, was lower for the medium concentration buffer (4,800 CFU/100 ml), which is 521. The lowest extent of relative growth recorded was observed for the highest concentration buffer (57,500 CFU/100 ml) at a value of 432.

Figure 4-9, on the other hand, shows that the increase in bacterial cells reaches is highest for the medium buffer concentration of 4,000 CFU/100 ml during the winter with a peak relative cells value of 2851. This is much higher than the extent of growth in case of the lower concentration buffer (555 CFU/100 ml) indicated from the maximum relative cells value of 212. The application of the high concentration buffer (43,500 CFU/100 ml) showed the lowest increase indicated from the maximum relative cells value of 48. It can also be noticed from **Figure 4-9** that the change in the case of the high bacterial concentration buffer at low temperature in the soil with 0.3% O.C. follows the same pattern as that in the 0.035% O.C. soil. In both cases, the increase occurs only within the first 24 hr period after which the bacterial cells start decreasing. Both **Figures 4-7** and **4-9** indicate that at low temperature the decay of the bacterial cells is a slow decay that may extend to a time frame of 7 days or more, especially in the lower range of initial buffer concentrations (500 and 5000 CFU/100 ml).

4.3.3 Seasonal Experiments Using Soil of High Organic Content

The final set of the bacteria survival tests were done on the same AUC soil after adding horse manure to increase its organic content from 0.035% to 0.5%. Seven columns of the soil, with this new organic content, were exposed to the same ambient outdoors atmosphere with the same experimental setup. The average concentrations of the prepared solutions ranged between 30,000 CFU/100 ml and 65,000 CFU/100 ml instead of the value 50,000 CFU/100 ml, between 3500 CFU/100 ml and 7500 CFU/100 ml instead of an exact value of 5000 CFU/100 ml, and between 600 CFU/100 ml and 700 CFU/100 ml instead of 500 CFU/100 ml.

The results show that the general trend for this set is an increase in the extent of growth of the bacteria during the high temperature season (the value of the bacterial cells is higher than that observed in the experiments done on soil with 0.3% organic content), and the duration for this growth is only 24 hours, followed by a rapid decay rate, which is consistent with the expected scenario for the pattern of survival in the presence of high temperature and nutrition in the soil. During the low temperature season, however, both the increase in bacterial population and the duration taken for the growth vary greatly according to the initial concentration.

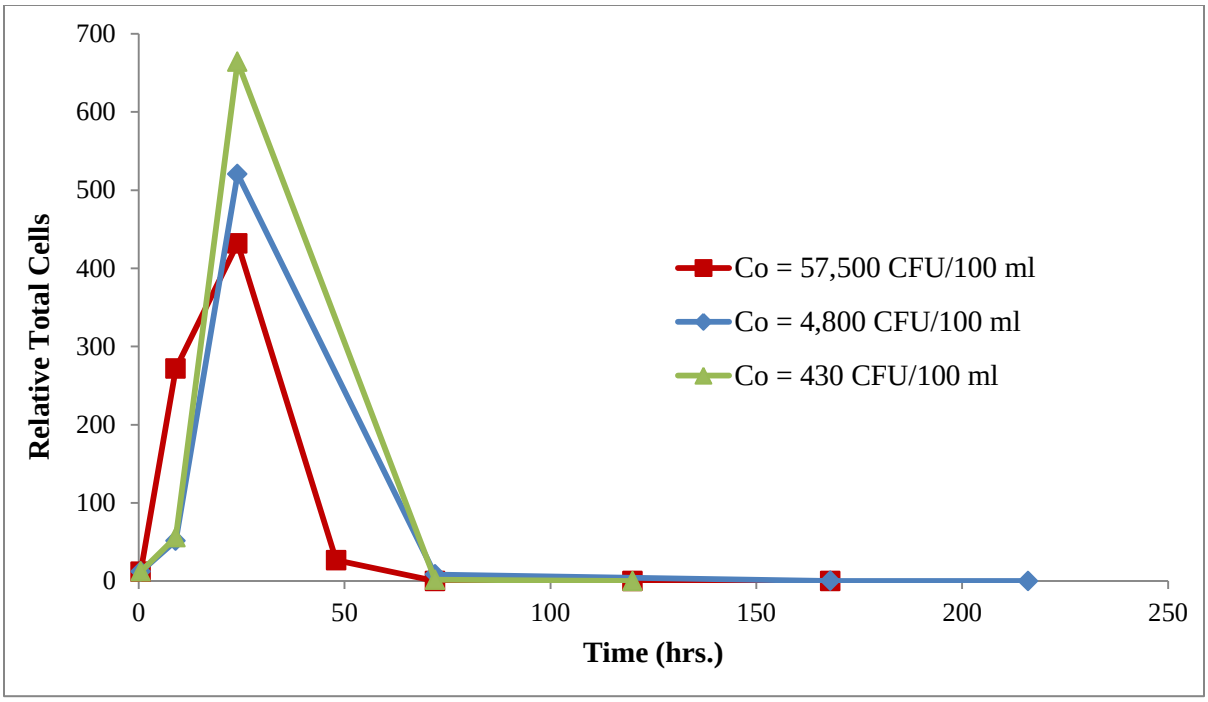

Figure 4-8 - Relative Total Cells vs. Time with 0.3% O.C. Soil at High Temperature for Various Initial *E-coli* concentrations

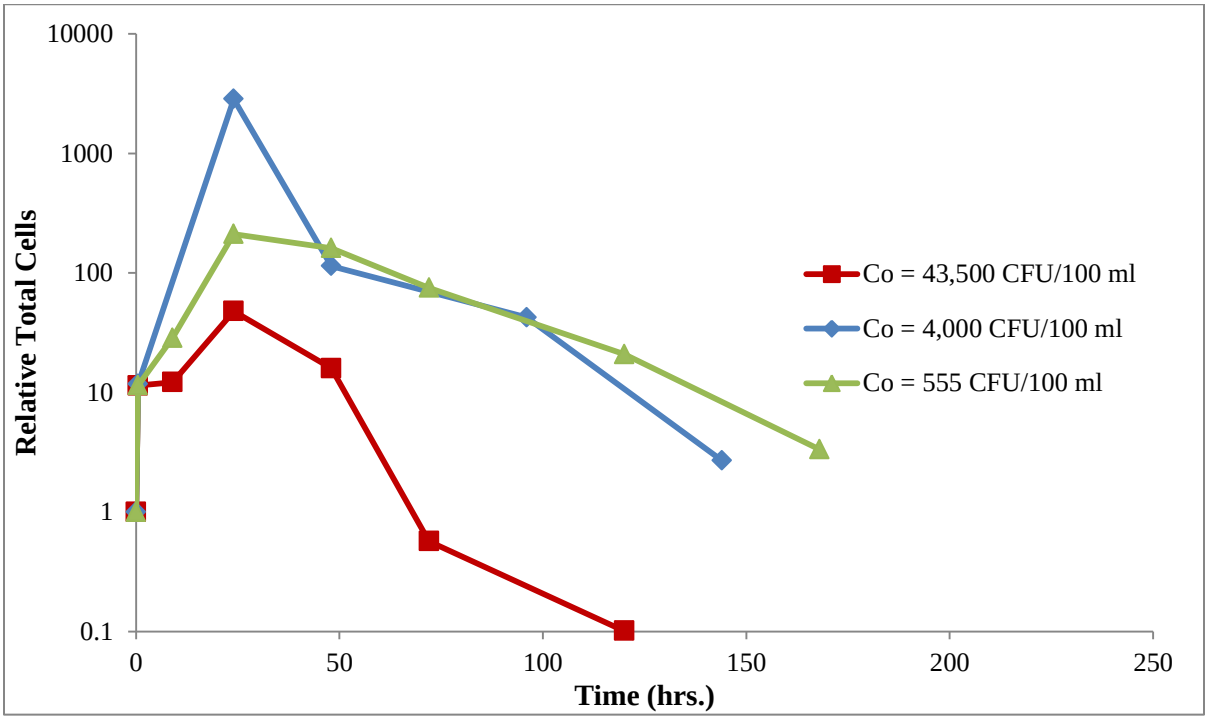

Figure 4-9 - Relative Cells vs. Time with 0.3% O.C. Soil at Low Temperature for Various Initial *E-coli* concentrations

Table 4-13 - Total and Relative Cells Values at for 0.3% O.C. Soil at High Temperature with High Concentration Buffer (~ 50,000 CFU/100 ml)

Depth (cm)		0.5	1.5	2.5	3.5	4.5	Total Cells
Time (Hour)	0.5	149,834	86,746	102,518	70,974	39,430	449,502
	9	2,193,180	1,581,748	1,827,650	3,289,770	1,608,332	10,500,680
	24	3,233,000	3,233,000	3,491,640	3,620,960	3,103,680	16,682,280
	48	137,938	206,268	232,450	254,163	197,966	1,028,785
	72	1,273	1,528	509	509	255	4,074
	120	0	0	159	159	0	318
	168	0	0	159	159	0	318
Depth (cm)		0.5	1.5	2.5	3.5	4.5	Relative Cells
Time (Hour)	0.5	4	2	3	2	1	12
	9	57	41	47	85	42	272
	24	84	84	90	94	80	432
	48	4	5	6	7	5	27
	72	0	0	0	0	0	0
	120	0	0	0	0	0	0
	168	0	0	0	0	0	0

Table 4-14 – Total and Relative Cells Values at for 0.3% O.C. Soil at Low Temperature with High Concentration Buffer (~ 50,000 CFU/100 ml)

Depth (cm)		0.5	1.5	2.5	3.5	4.5	Total Cells
Time (Hour)	0.5	95,965	80,233	76,300	49,556	21,238	323,293
	9	94,041	91,255	75,929	54,335	29,257	344,817
	24	191,816	264,889	331,534	443,850	124,494	1,356,583
	48	53,841	83,717	146,094	89,626	77,807	451,084
	72	2,134	2,955	5,253	3,940	1,806	16,087
	120	325	260	1,692	390	195	2,863
	168	0	0	0	0	0	0
Depth (cm)		0.5	1.5	2.5	3.5	4.5	Relative Cells
Time (Hour)	0.5	3	3	3	2	1	11
	9	3	3	3	2	1	12
	24	7	9	12	16	4	48
	48	2	3	5	3	3	16
	72	0	0	0	0	0	1
	120	0	0	0	0	0	0
	168	0	0	0	0	0	0

Figure 4-10 indicates that during the summer, highest bacterial growth occurs both when the concentration of the buffer is as high as 65000 CFU/100 ml, and as low as 675 CFU/100 ml. In both cases, the value of the relative cells reaches approximately 1000 after 24 hours. The lowest extent of growth as indicated from the diagram occurs upon the application of a medium buffer concentration of 7500 CFU/100 ml, for which the value of the relative cells is 211 after 24 hours. This result seems odd versus all of the previous cases and may be an anomaly.

For the winter season (10 – 21 °C), **Figure 4-11** shows that the trend very much resembles that presented in **Figure 4-7** for the "as is" soil at lower temperature, in which the growth period of the *E-coli* bacteria in the soil is extended to 3 – 5 days until reaching the maximum. Also, the extent of increase upon the application of the lower range buffer concentrations (630 and 3550 CFU/100ml) is high relative to the high initial concentration case. The relative cells in the case of the low concentration buffer reaches a value of 792 after 3 days (72 hours) after which it starts decaying, while the relative cells for the medium concentration buffer application reaches a value of nearly 5000 during a time frame of 5 days, after which it starts decaying. When a bacterial buffer solution of a concentration as high as 30,000 CFU/ 100 ml is applied to the soil, the relative increase is just over 10 followed by a slow decay (which is the same as what happened in the case of the 'as is' soil).

4.3.4 Effect of Temperature on Bacteria Survival in Soil

In general, the results of the bacteria survival tests suggest that the winter season is more favorable for bacterial growth, which is consistent with a previous study on bacterial and fungal growth in soil. The findings of this study showed that the bacterial population survives less at high temperature seasons, whereas fungi survived less during the winter (Pietikåinen, Pettersson, & Bååth, 2005). The results from the bacteria survival tests conducted for different soil organic fractions and with different initial concentrations, such as those presented in the retrieved concentrations in **Table 4-6** and **Table 4-7** for the 'as is' soil, and the values of the total and relative cells presented in **Table 4-13** and **Table 4-14** for the 0.3% organic fraction soil, are consistent with those previous findings. Also, the results presented in these tables indicate that the change in the number of cells in the soil column not only occurs with time, but also with depth, and the latter can be observed after a specific duration for the same soil type (such as the 'as is' soil after 24 hours).

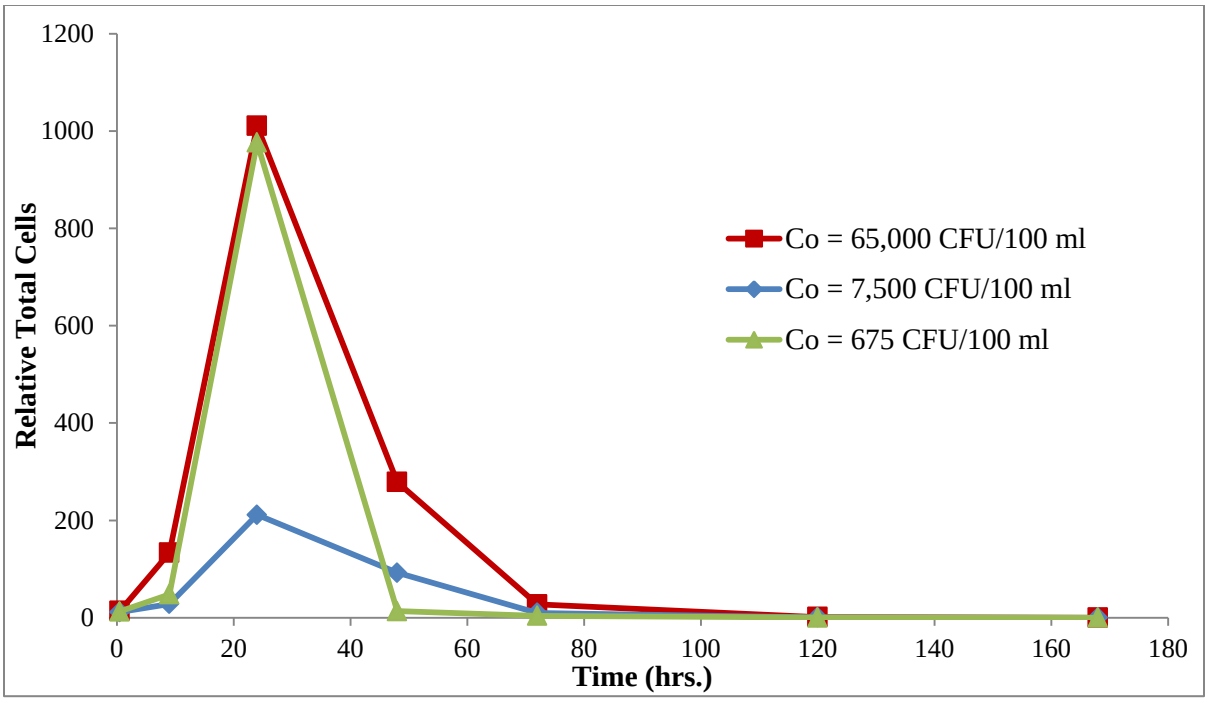

Figure 4-10 - Relative Total Cells vs. Time with 0.5% O.C. Soil at High Temperature for Various Initial *E-coli* Concentrations

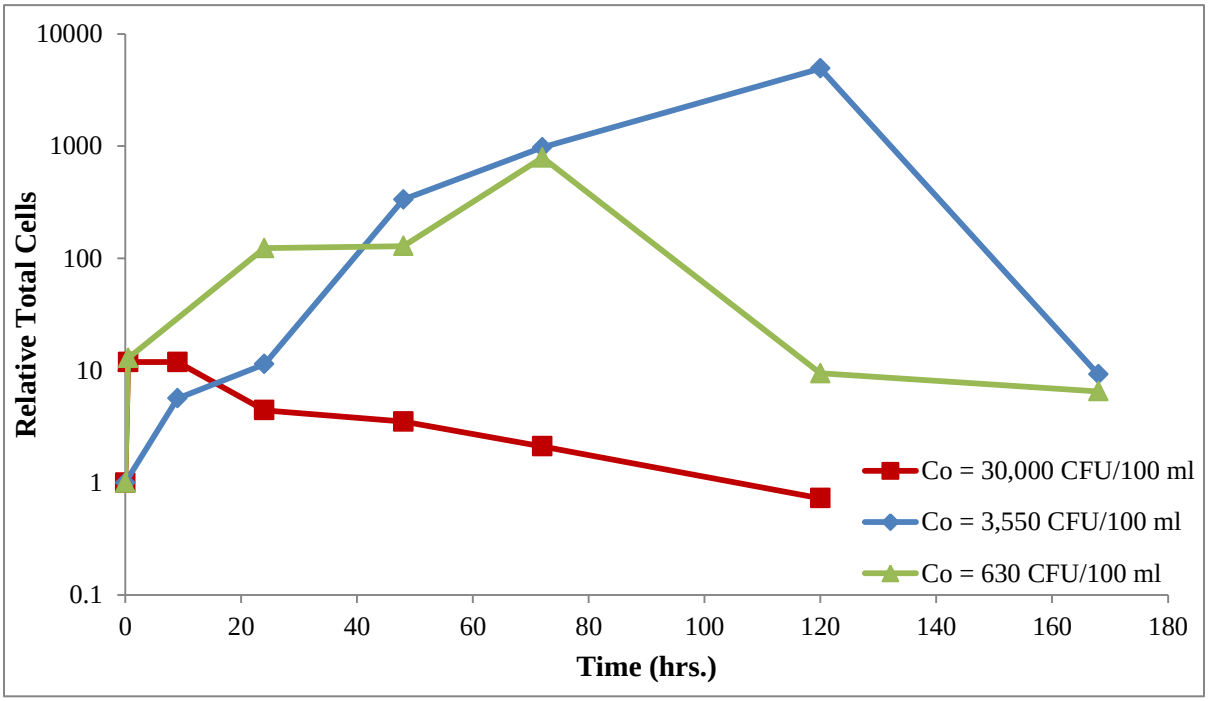

Figure 4-11 - Relative Total Cells vs. Time with 0.5% O.C. Soil at Low Temperature for Various Initial *E-coli* Concentrations

Change with Temperature According to Initial Concentration

Temperature can either directly impact the survival pattern of the bacteria in the soil, or it can affect this pattern indirectly by affecting the soil moisture as well as the chemical and physical characteristics of the soil. The results expressed in the previous tables and figures show how the difference in temperature is an important factor that affects the growth rate of the bacteria and the extent of this growth, which can be easily determined through the values of the total cells in the column as well as the value of relative total cells. The season that is most favored for bacterial growth in terms of the extent of growth (measured in concentration or total cells) seems to be the winter season with less heat and sunlight as well as frequency of rainfall, all of which contribute to increasing the soil moisture.

The exception to this was the application of the high concentration buffer for which the extent of bacterial growth (from the value of either the relative cells or the total cells in the entire column) was favored during the summer much more than the winter, regardless of the soil organic content. For example, the extent of growth in the 0.3% O.C. soil was almost 10 times greater in summer than in winter as indicated from **Figure 4-13**. This suggests that the primary contributing factor for a highly contaminated bacterial solution is the temperature.

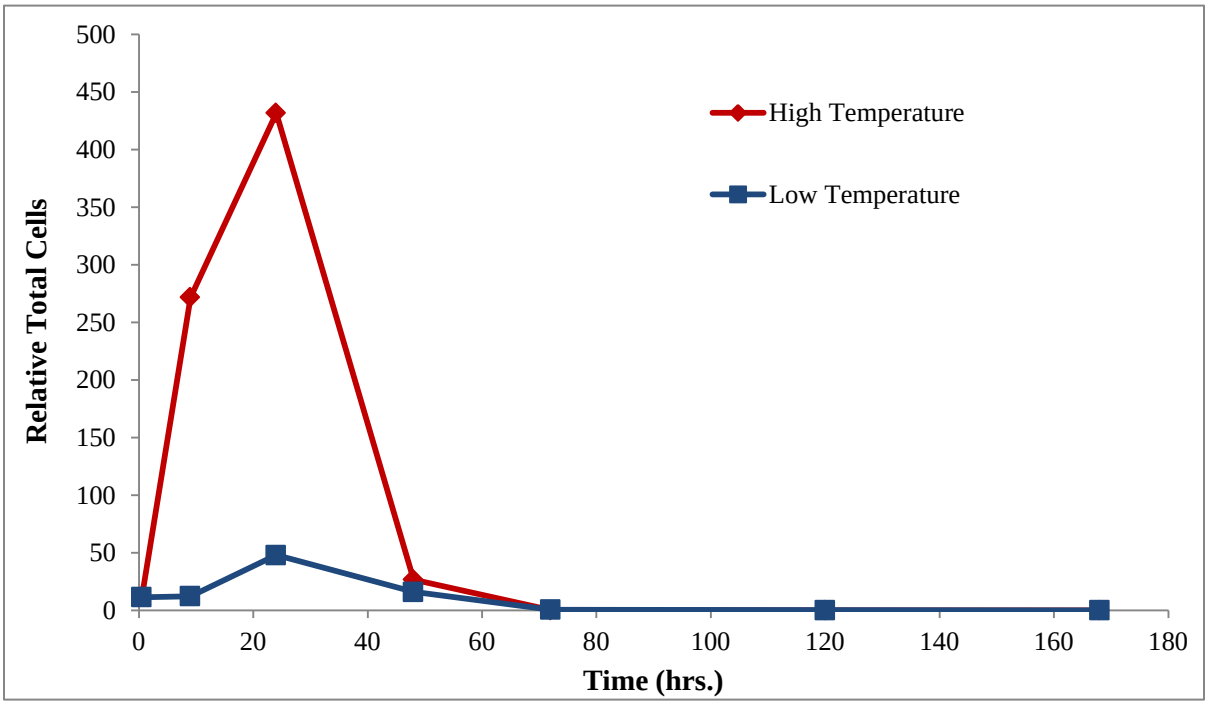

Figure 4-12 - Relative Cells vs. Time for High Concentration Buffer Applied to 0.3% O.C. Soil

Figure 4-13 and **Figure** 4-14 show that for the case of applying a buffer of medium concentration (in the range of 3,500 – 7,500 CFU/100 ml) to the soil, the number of cells increases in the winter much more than in the 0.5% O.C. soil. This pattern is similar to that observed in the 'as is' soil. **Figure 4-14** illustrates that although the same winter growth is experienced in the 0.3% organic fraction soil, there is a significant difference in that the growth peak achieved after just 24 hours like in summer and followed by a rapid die-off of viable cells. The winter growth period is not extended as with the "as is" and 0.5% soils and the reason why this difference occurred is not clear.

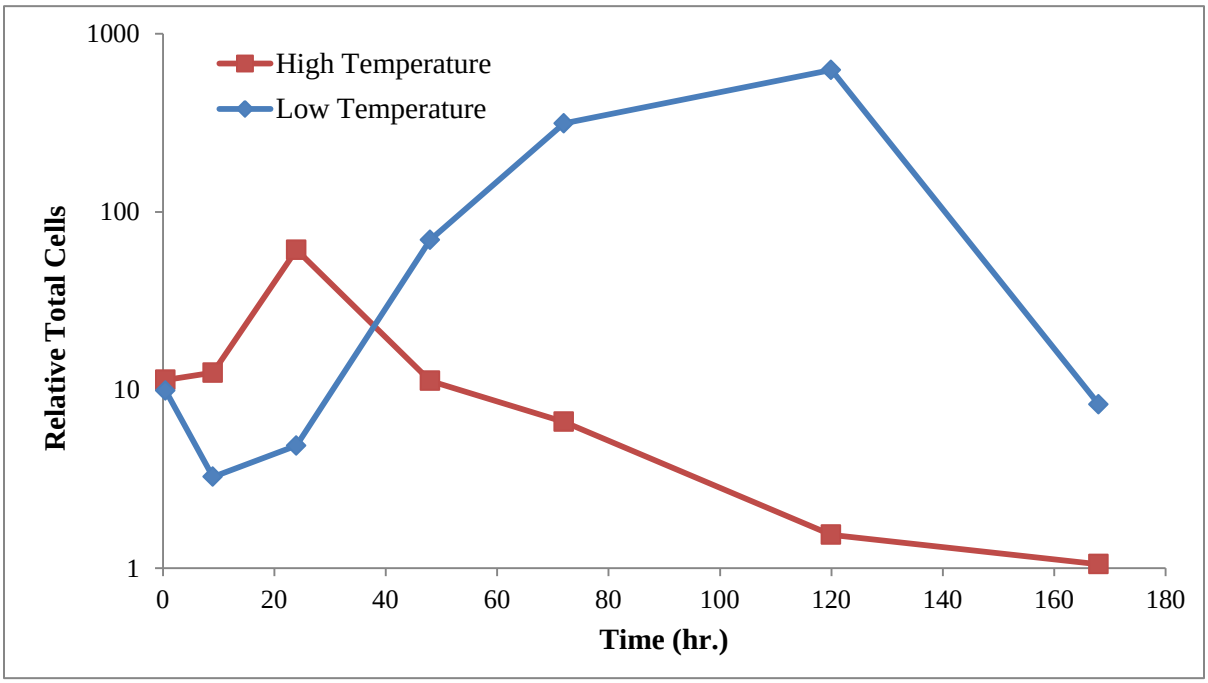

Figure 4-13 - Relative Cells vs. Time for Medium Concentration Buffer Applied to 'As Is' Soil

When a bacterial solution of a low concentration (within the range 400 – 700 CFU/100 ml) is applied, the *E-coli* population reaches its peak in a time interval of 24 hours during the summer season. This is true for all three soil organic fractions. Figure 4-16 and Figure 4-17 together with Table 4-14 indicate that this peak value increases with increasing soil organic content. The relative total cells increases from about 11 to 660 to nearly 1000 for 0.035%, 0.3%, and 0.5% carbon content, respectively. Each case is also characterized by very rapid decay. A consistent but unanticipated trend at low temperature is that for the 0.3% organic fraction, the peak production of cells occurs at about 1 day while for the low and high organic content cases the peak occurs at 3 or 4 days at lower temperature. Similar to the high temperature experiments, the relative total cells increases with the soil organic fraction (from 30 to 200 to 800 for 0.035%, 0.3%, and 0.5%, respectively).

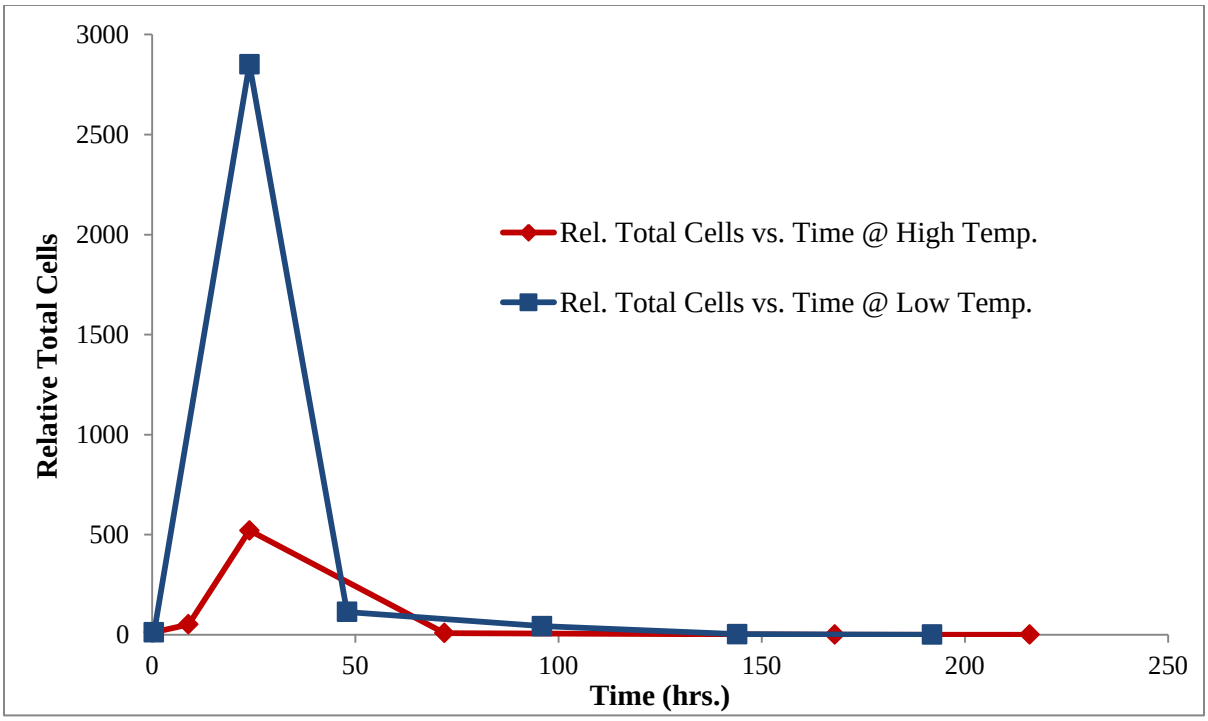

Figure 4-14 - Relative Cells vs. Time for Medium Concentration Buffer Applied to 0.3% O.C. Soil

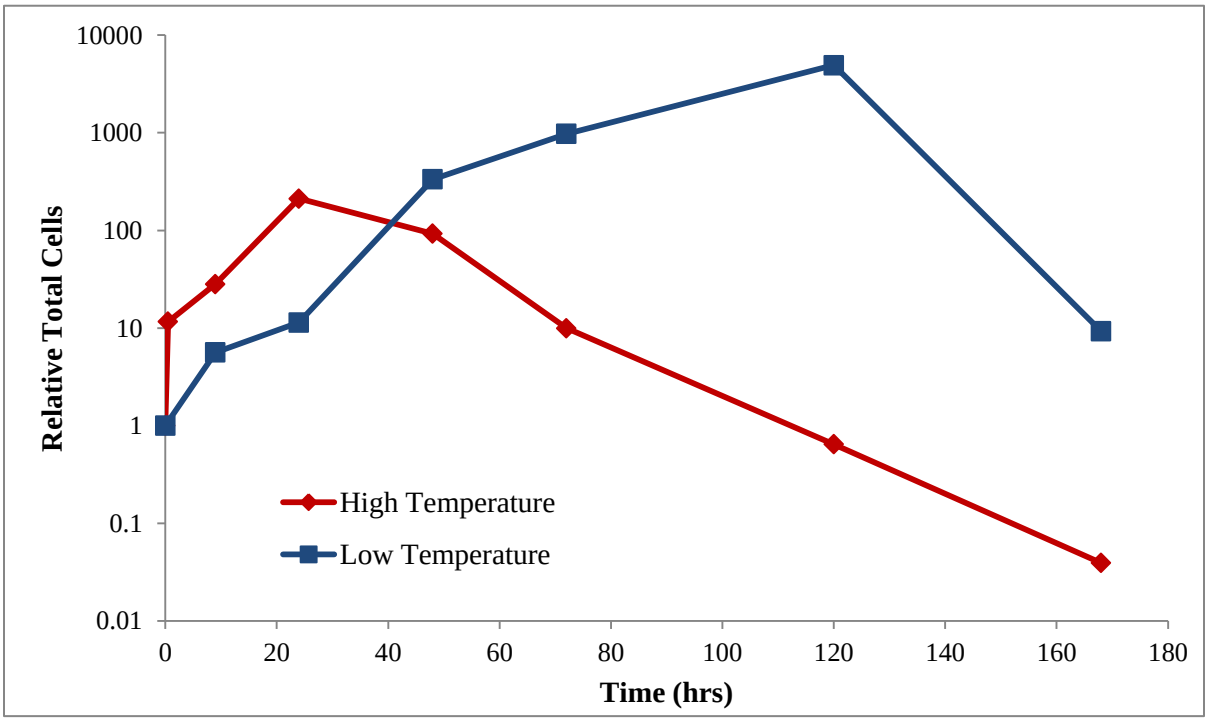

Figure 4-15 - Relative Cells vs. Time for Medium Concentration Buffer Applied to 0.5% O.C. Soil

The observed trends for low initial concentration confirm that the extent of bacterial growth is enhanced by the presence of organic matter in the soil matrix. They also indicate that in most but not all cases, the growth rate is slower (*i.e*. the peak biomass production occurs later) and the growth period prolonged at cooler temperature. This is likely due to increased drying at the surface at high temperatures in contrast to moisture retention prolonging cell life at lower temperature. The prolonged growth at lower temperature is also due to less intense solar radiation (especially ultraviolet radiation) which is known to be effective for coliform inactivation (Manios, Maraitaki, & Mantzavinos, 2006; O'Toole, Sinclair, Diaper, & Leder, 2008)

These results are also consistent with previous research findings which demonstrated that the die-off rate for fecal coliforms is more rapid during the summer (Foppen & Schijeven, 2006; Van Donsel, Geldreich, & Clark, 1967): For example, 90% decrease of fecal coliform was observed after 3.3 days in summer as opposed to 13.4 days in autumn. Van Donsel, *et al*. (1967) also presented a value of the die-off rate constant k= 0.294 day^{-1} for fecal coliforms in soil at a field exposed to sunlight as opposed to the value 0.133 day^{-1} for a shaded field in summer. For the same shady area, the value of k obtained in the winter was 0.191 day^{-1} for a field exposed to sunlight as opposed to 0.075 day^{-1} for a shaded field, which indicates that the bacteria survival is greatly dependent on both temperature and sunlight exposure.

Table 4-15 - Relative Cells for Medium Conc. Buffer Applied to Soil As Is

Relative Cells For Med Conc. Buffer at High Temperature							
Depth (cm)		**0.5**	**1.5**	**2.5**	**3.5**	**4.5**	**Rel. Cells**
Time (Hour)	**0.5**	0	1	2	3	5	11
	9	1	1	2	3	5	13
	24	1	6	7	17	30	61
	48	0	1	2	3	6	11
	72	1	1	1	1	3	7
	120	1	0	0	0	1	2
	168	0	0	0	0	1	1
Relative Cells For Med Conc. Buffer at Low Temperature							
Depth (cm)		**0.5**	**1.5**	**2.5**	**3.5**	**4.5**	**Rel. Cells**
Time (Hour)	**0.5**	2	2	2	3	1	10
	9	1	1	0	1	0	3
	24	1	1	1	1	0	5
	48	27	9	15	9	10	70
	72	1	108	53	58	94	314
	120	17	213	140	218	38	626
	168	7	0	0	0	1	8

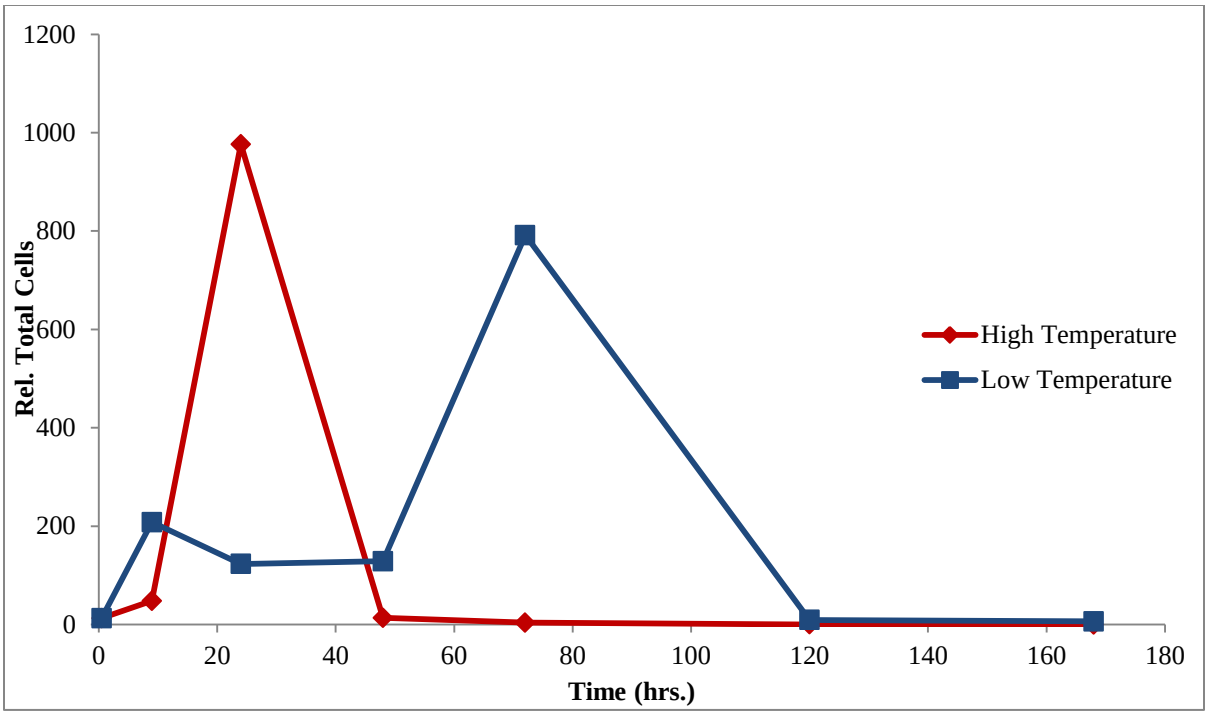

Figure 4-16 - Relative Cells vs. Time for Low Concentration Buffer Applied to 0.5% O.C. Soil

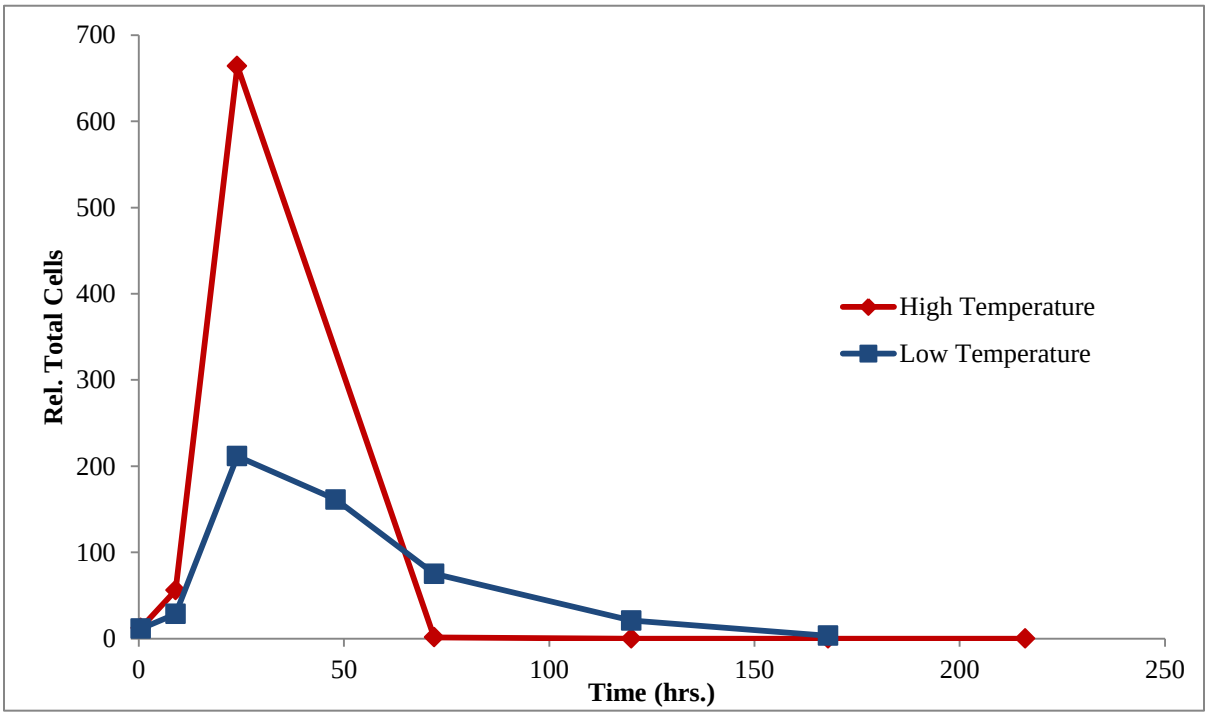

Figure 4-17 - Relative Cells vs. Time for Low Concentration Buffer Applied to 0.3% O.C. Soil

Change with Depth According to Initial Concentration

The results also indicate that, depending on the temperature and atmospheric conditions (sunlight, rain *etc.*) and the initial concentration of the bacterial solution applied to the soil, the bacteria survival pattern changes with depth. The results also suggest that there can be a depth preference for the bacteria survival in that during both seasons, the highest bacterial population was observed at a depth 3.5 cm (away from the surface), in many of the cases studied. During the summer, the bacterial cells decrease from the top layer of the soil more gradually such as in the case of the 0.3% organic content soil, as shown in **Table 4-13** and **Table 4-14,** where the highest bacterial concentration can be found away from the surface of the soil and at a depth of 3.5 cm during the summer, and within the 2.5 – 3.5 cm range during the winter.

In the case of AUC soil "as is" (organic fraction = 0.035%), the contributing factors to the growth of bacteria are mainly the atmospheric conditions (temperature and sunlight intensity). The results of this particular set show that during the summer, during which time the soil columns are exposed to more intensive solar radiation and high temperatures (30 - 35°C or more), the bacterial cells were found to mainly increase away from the top layer of the soil columns – that is, the relative bacterial cells increase with depth, and are maximum at the 3.5 and 4.5 cm depths as shown in **Figure 4-18** through **Figure 4-21**.

These concentration profiles illustrate that during the summer, the relative total bacterial cells are lowest at the surface of the soil regardless of the organic content of the soil. Although the initial concentration of the buffer solution is the same for all soils and at *t=0*, the relative total cells change with time along the soil columns indicating the transport of the bacteria to more favorable depths as well as the noticeable change in the die-off rate along the soil column. The peak value, as shown in the figures (**Figure 4-20** and **Figure 4-21** show that the bacterial cells at a large depth increase up to three orders of magnitude from the initial value and most noticeable at a depth of 3.5 cm), in most cases occur after 24 hours from irrigation and at such a time, the highest number bacterial cells can therefore be found at larger depths, possibly because at these depths there is less aeration and therefore less decomposition of the organic content existing in the soil. Another factor can also be related to water drainage to these depths and the higher rate of evaporation at the surface due to the heat and radiation. At these depths (3.5-4.5), in particular, the die-off rate for the bacterial cells may be slow for the same reasons just explained.

The use of reclaimed wastewater with bacterial contamination equal to or higher than 3500 CFU/100 ml is therefore accompanied by health risks. To reduce such risks to workers, for example, it is advisable for them to have limited contact with the topsoil after irrigation for at least 48 hours in the summer and 4-5 days in the winter until bacteria decay has achieved a significant level.

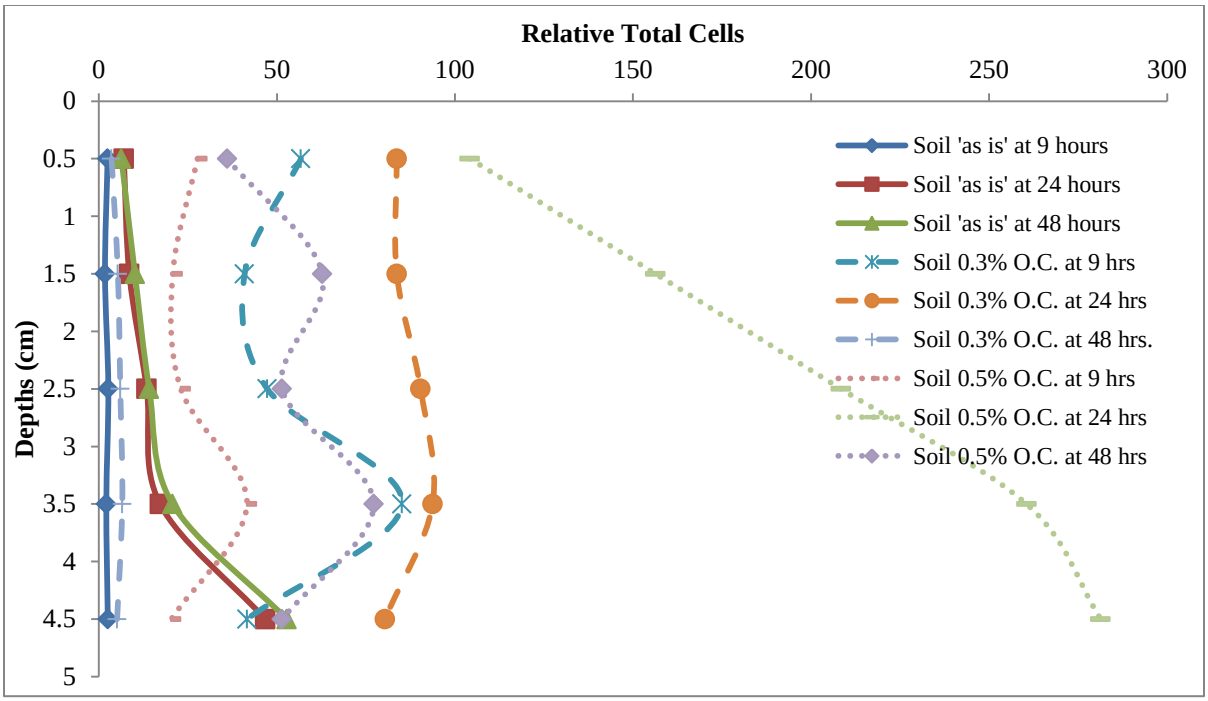

Figure 4-18 - Bacteria Growth Pattern along the Soil Column at Different Time Intervals for High Conc. Buffer at High Temperature

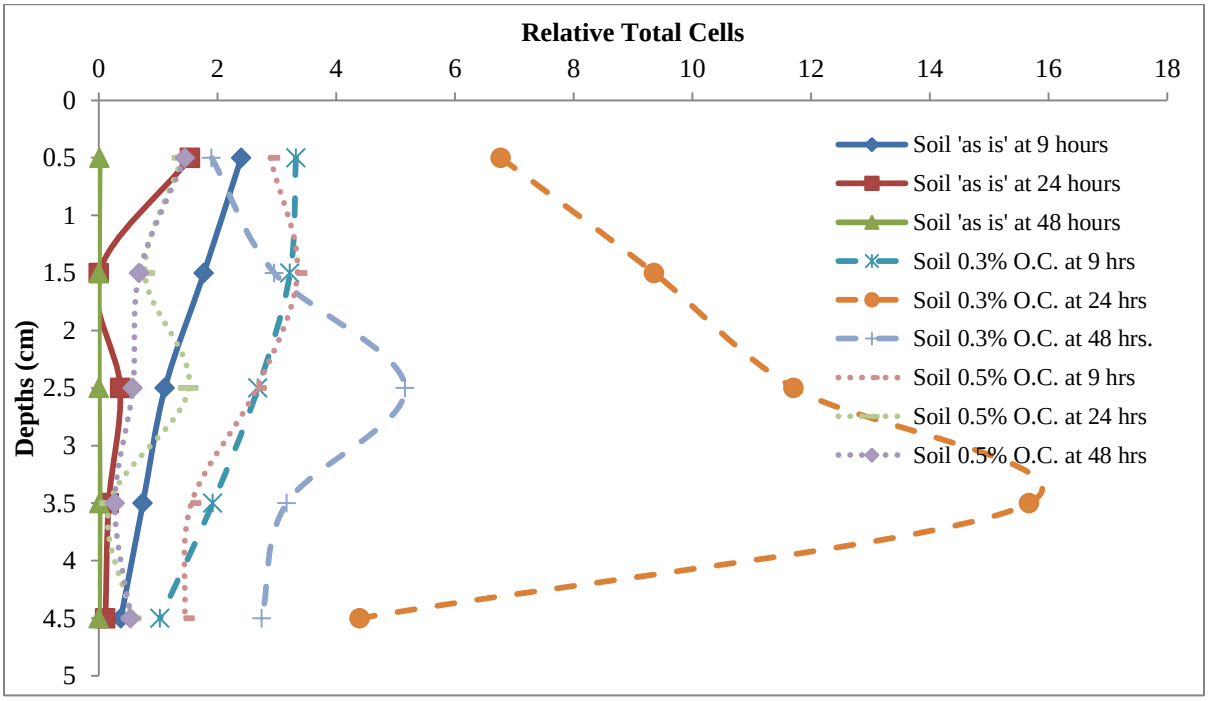

Figure 4-19 - Bacteria Growth Pattern along the Soil Column at Different Time Intervals for High Conc. Buffer at Low Temperature

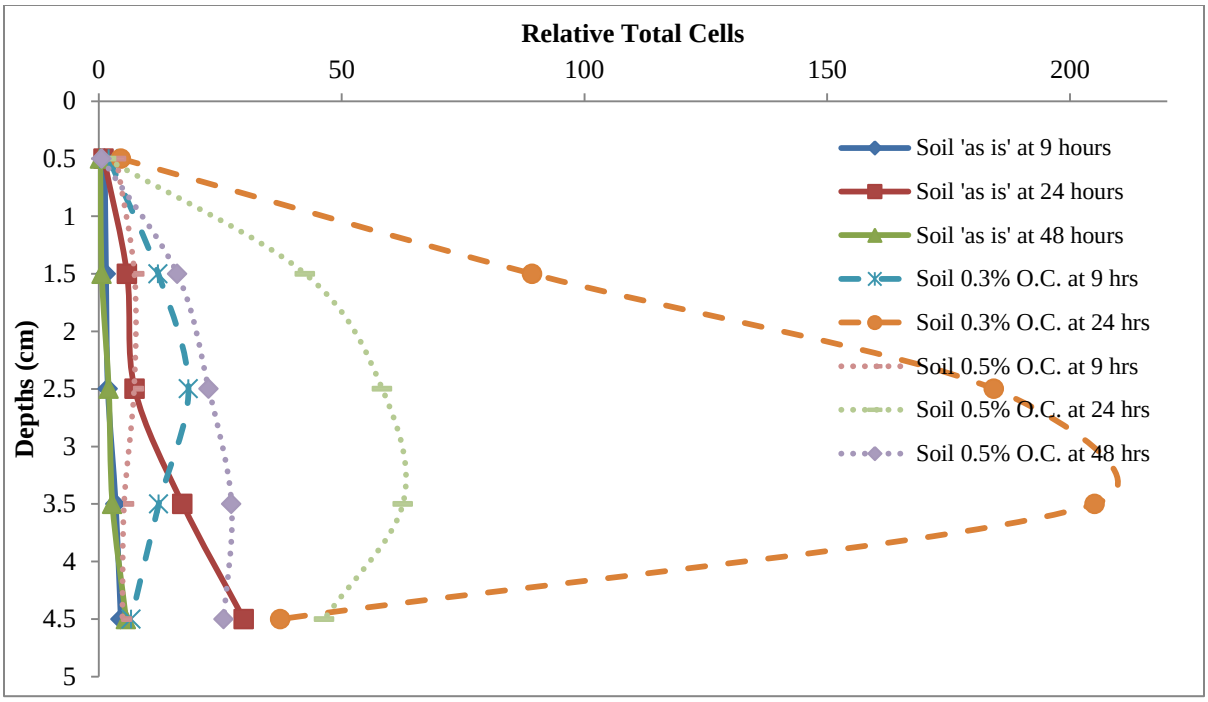

Figure 4-20 - Bacteria Growth Pattern along the Soil Column at Different Time Intervals for Medium Conc. Buffer at High Temperature

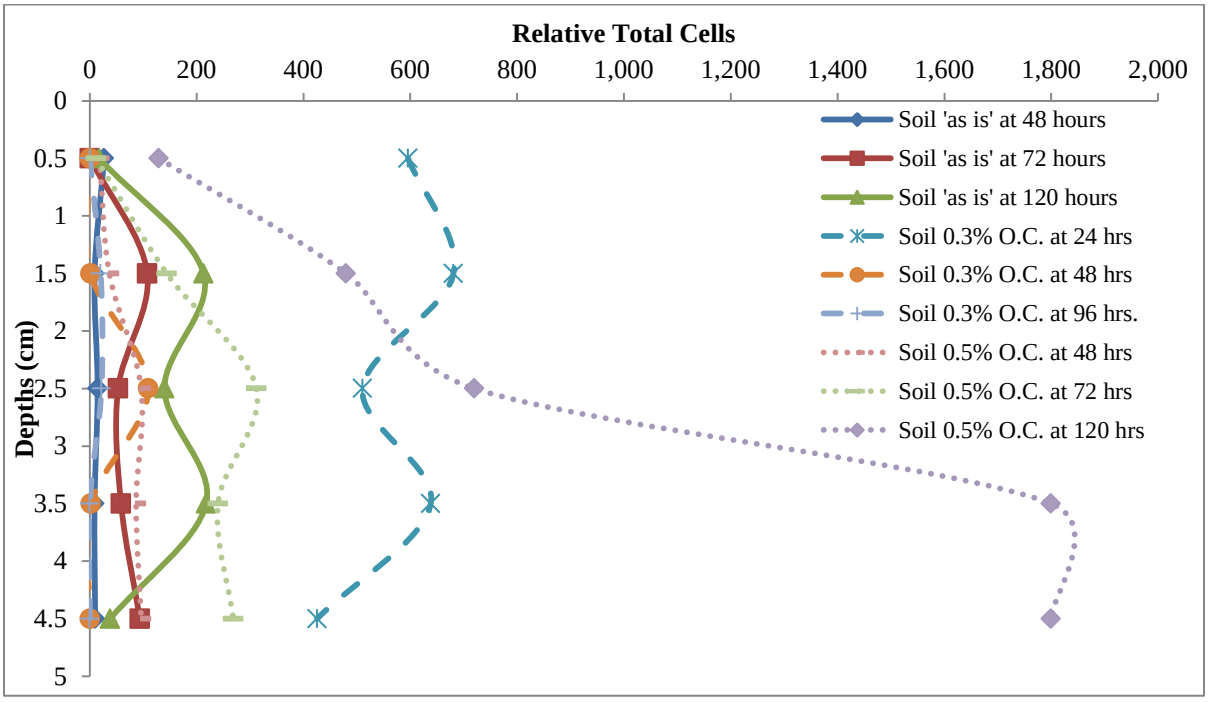

Figure 4-21 - Bacteria Growth Pattern along the Soil Column at Different Time Intervals for Medium Conc. Buffer at Low Temperature

4.3.5 Effect of Organic Content on Bacteria Survival in Soil

What was expected even before adding the organic content to the soil in this experiment is an increase in the *E-coli* relative to the initial concentration added. What was also expected was an increase in the total concentration of the *E-Coli* in a column with the increase in the organic content. What was observed from the experiments, however, was different. **Figure 4-22** shows that during the summer and upon the application of a medium concentration buffer, the survival (growth and decay rates) of bacteria follows almost the same pattern reaching the maximum growth (as indicated by the values of the relative cells) at 24 hours. However, the figure shows that the highest relative total bacterial cells value (as well as highest value of total cells in the column) is reached at an organic content (O.C.) 0.3% (*i.e.* Relative Total Cells at 0.3% O.C. > Relative Total Cells at 0.5% O.C. > Relative Total Cells at 0.035% O.C.).

The growth pattern for each soil O.C. can also be observed in **Figure 4-23** which represents the total bacterial cells recovered from each column plotted against time during their growth period. The diagram shows that the growth of the bacterial cells can be characterized by means of a zero-order linear growth, in which the total cells of the bacteria in the entire column is directly proportional to time and with R^2 values ranging between 0.87 – 0.885, which indicates a good fit. The decay period was also plotted and represented by first-order exponential fit shown in **Figure 4-24**. The plot starts from the maximum value reached during the growth period and the time at which this value is reached (24 hours in this case), and continues until the minimum value is reached. The diagram shows that this fit is much better than the zero-order growth, for which the values of the R^2 range between 0.9 and 0.99. These results are consistent with the findings from Badawy's study (Badawy, 2005).

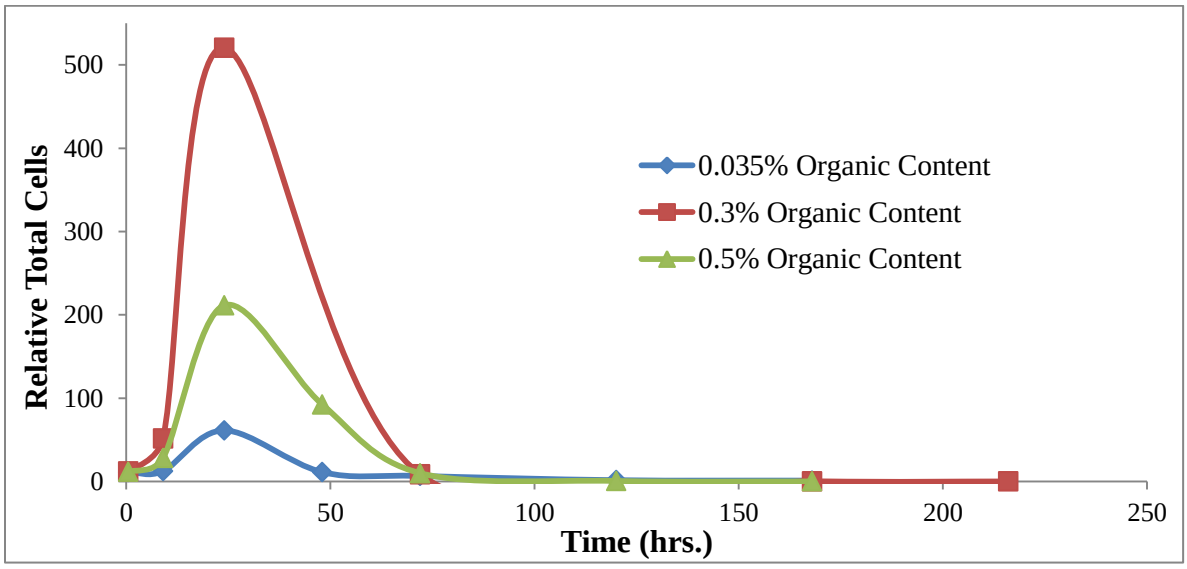

Figure 4-22 - Relative Cells vs. Time for Different Soil Organic Content Using Medium Concentration (~5000 CFU/100 ml) Buffer and at High Temperature

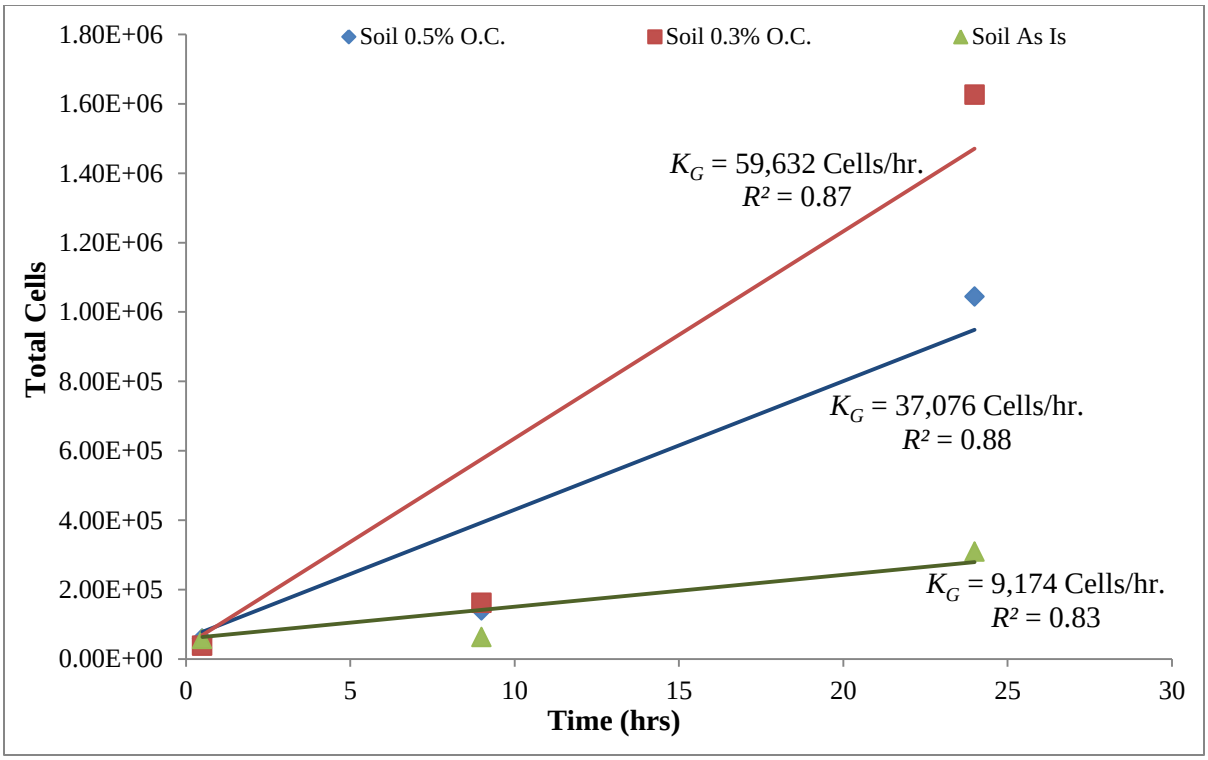

Figure 4-23 - Zero Order Growth of Total Bacterial Cells for Different Organic Content Soils Using Medium Concentration (~5000 CFU/100 ml) Buffer and at High Temperature

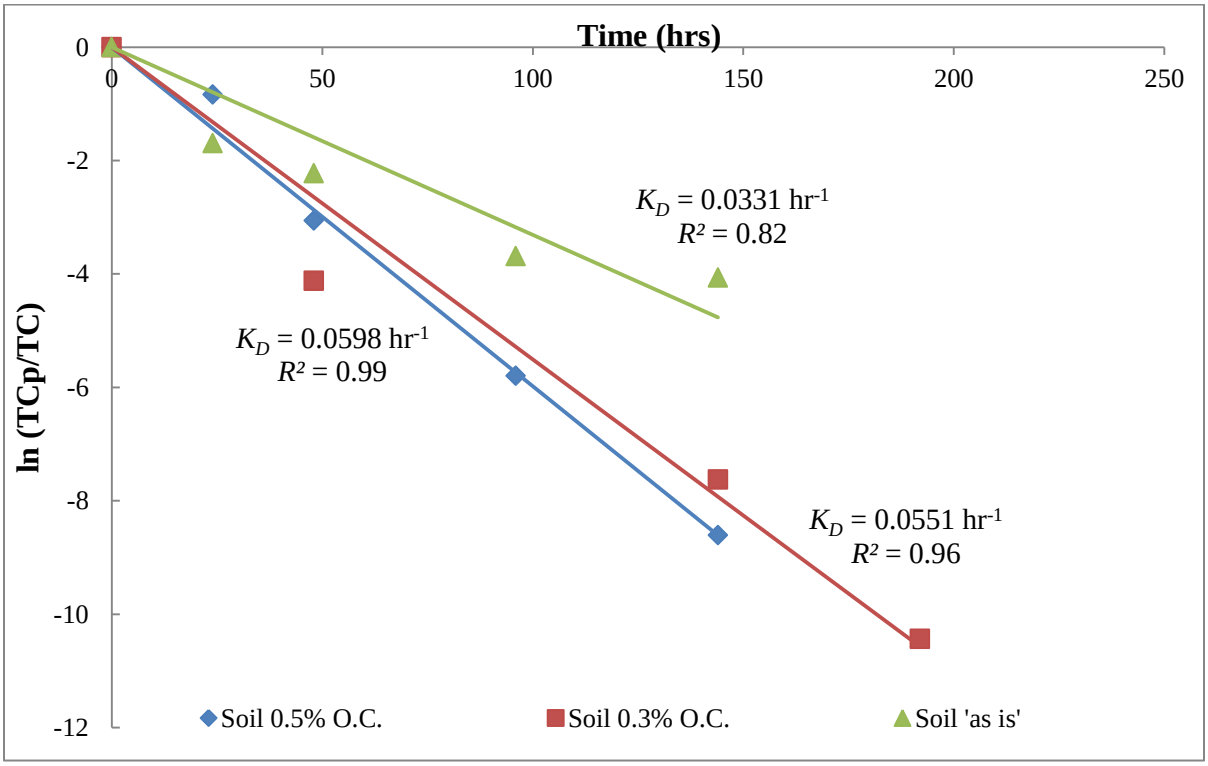

Figure 4-24 - Exponential Decay of Total Bacterial Cells for Different Organic Content Soils Using Medium Concentration (~5000 CFU/100 ml) Buffer and at High Temperature

Figure 4-22 shows that for the winter experiments using the same buffer concentration, the survival (growth and decay rates) of bacteria follow a different pattern for the different organic fractions. First, the maximum total cells is not reached at the same time for the three cases: for the 0.3% O.C. soil, the bacteria reached its peak at 24 hours followed by rapid decay. On the other hand, the peak is reached for the 0.035% and 0.5% soil O.C. at 120 hours, which is expected at lower temperature. The order, in terms of the relative total cells values, was observed in this case as: 0.5% O.C. > 0.3% O.C. > 0.035% O.C. This trend is consistent with the notion that biomass production is directly proportional to the soil organic content.

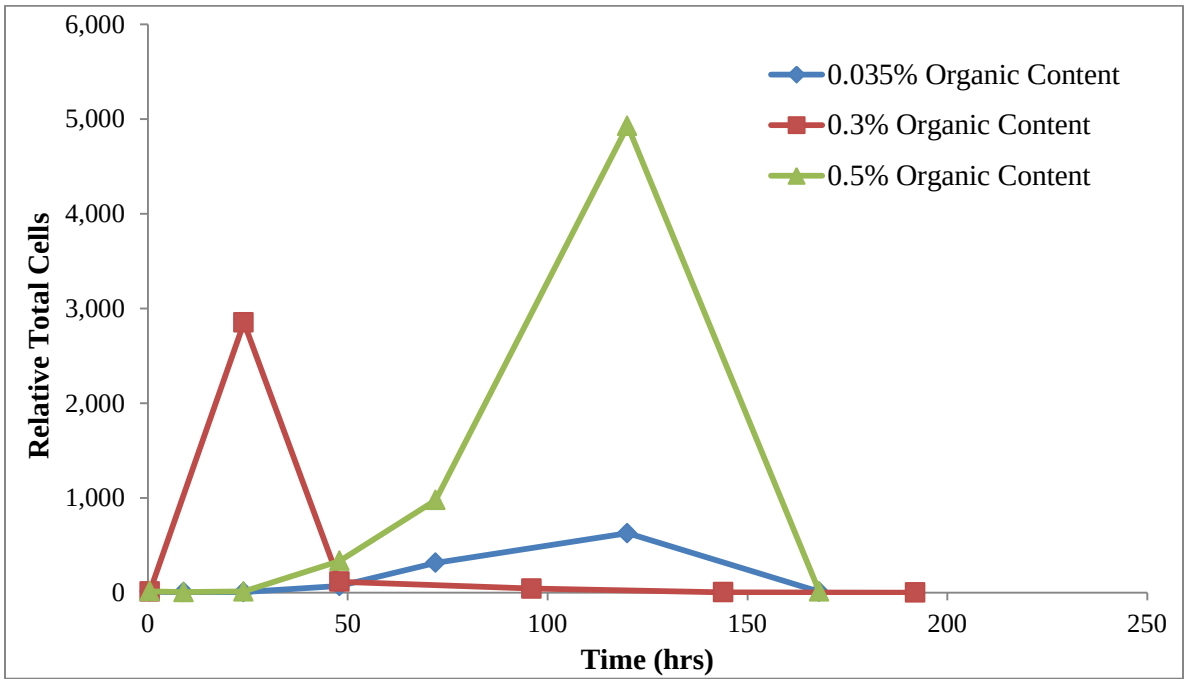

Figure 4-25 - Relative Cells vs. Time for Different Organic Content Soils Using Medium Concentration (~5000 CFU/100 ml) Buffer at Low Temperature

The change in the growth pattern for the experiments depicted in **Figure 4-25** can also be observed in **Figure 4-26**, which also represents the total bacterial cells recovered from each column in this set plotted against time. The growth of the bacterial cells was characterized by means of a zero-order growth model, in which the total cells of the bacteria in the entire column is directly proportional to time and with R^2 values ranging between 0.72 – 0.999. The decay period was also plotted and represented by first-order decay shown in **Figure 4-27**. The diagram was based on the typical assumption that the decay rate of the bacteria is represented by a first order decay model despite the scarcity of the data that would correctly represent it. Therefore, in some cases, there may only be two data points, namely the peak value and one subsequent non-zero value. This follows the approach previously used by Badawy (2005).

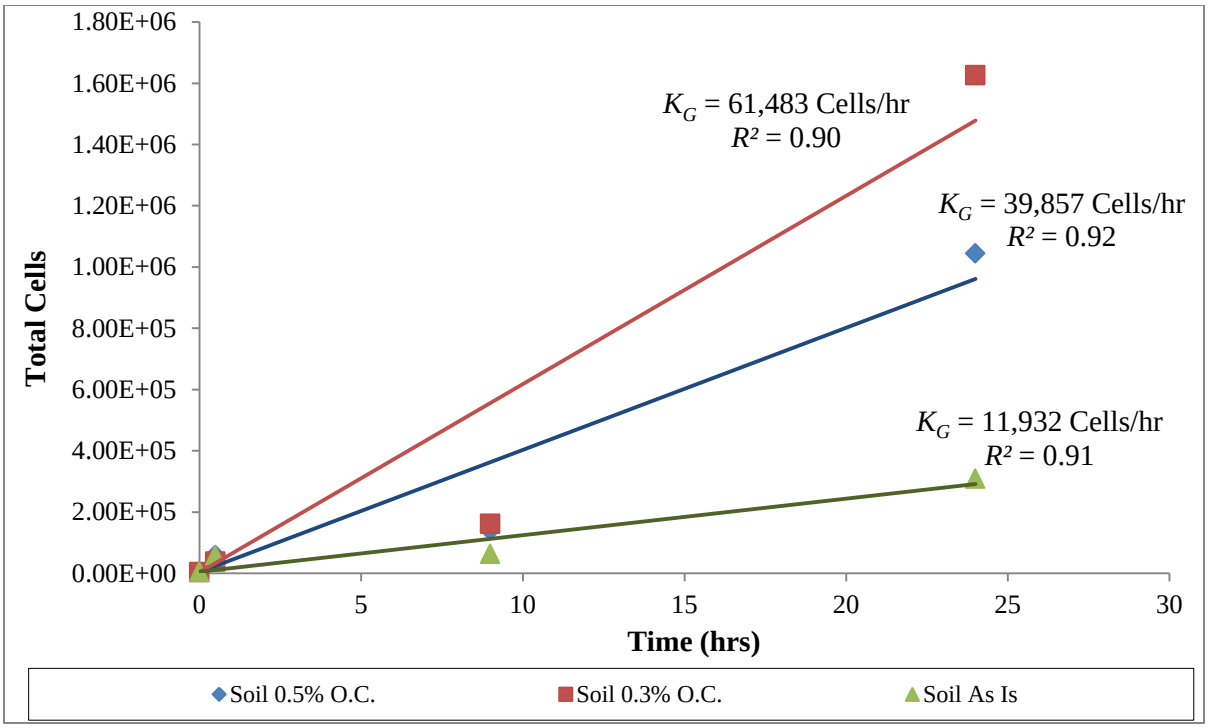

Figure 4-26 - Zero Order Growth of Total Bacterial Cells for Different Organic Content Soils Using Medium Concentration (~5000 CFU/100 ml) Buffer and at Low Temperature

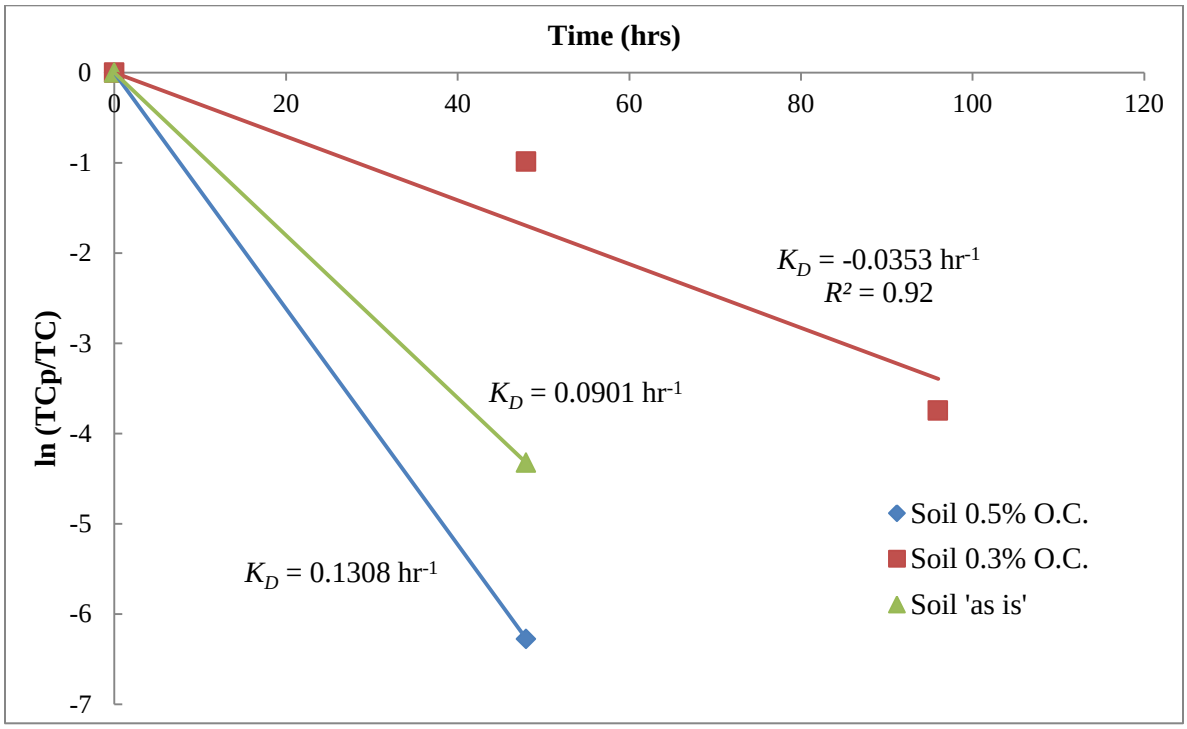

Figure 4-27 - Exponential Decay of Total Bacterial Cells for Different Organic Content Soils Using Medium Concentration (~5000 CFU/100 ml) Buffer and at Low Temperature

4.4 Estimation of Growth and Decay Rates

The considerable variations in the experimental results is related in part to the complex matrix of variables studied; namely soil organic content, temperature and sunlight exposure, and initial concentrations. It may also be attributed to experimental error, an unrecorded change in weather, or other natural processes that are difficult to account for in dealing with living systems. The growth of the bacteria in soil, in most cases, may be assumed to follow a zero-order (linear) model, with *Total Cells (TC)* = K_G *(t)* + TC_o *(*where K_G represent the growth constant in total cells/hr., and TC_o is the fixed intercept equal to the total cells added to the column in one pore volume of buffer solution at $t = 0$). **Table 4-16** is a compilation of the zero-order growth constants for all 18 experiments.

Table 4-17 displays all of the estimated decay rate values from the expression: $TC\ (t) = TC_p e^{-KDt}$, where TC_p is the total cells at the peak value, while K_D is the decay rate measured in 1/hr. It follows from this equation that $\ln(TC/TC_p) = -K_D t$

Table 4-16 - Estimation of Growth Rates from Zero-Order Growth Model

	Org. Fraction	Equation	K_G (Total Cells/hr)	R^2
High Conc. Buffer - High Temp.	0.50%	TC = 1,661,536 t + 39,650	1,661,536	92.4
	0.30%	TC = 751,394 t + 37,375	751,394	92.1
	0.04%	TC =74,891 t + 30,150	74,891	94.0
Mid. Conc. Buffer - High Temp.	0.50%	TC = 39,857 t + 4,575	39,857	92.0
	0.30%	TC = 61,483 t + 3,120	61,483	90.3
	0.04%	TC = 11,932 t + 5,025	11,932	91.2
Low Conc. Buffer - High Temp.	0.50%	TC =14,958 t + 412	14,958	87.0
	0.30%	TC = 6,991 t + 280	6,991	89.4
	0.04%	TC = 7,771 t + 362	7,771	N/A
High Conc. Buffer - Low Temp.	0.50%	TC = 22,193 t + 18,300	22,193	N/A
	0.30%	TC = 53,061 t + 28,319	53,061	90.0
	0.04%	TC = 517,710 t + 24,321	517,710	N/A
Mid. Conc. Buffer - Low Temp.	0.50%	TC = 65,208 t + 2,166	65,208	74.9
	0.30%	TC = 309,143 t + 2,604	309,143	100.0
	0.04%	TC = 10,834 t + 2,412	10,834	88.0
Low Conc. Buffer - Low Temp.	0.50%	TC = 4,299 t + 384	4,299	97.2
	0.30%	TC = 2,918 t + 361	2,918	92.1
	0.04%	TC = 190 t + 335	190	90.4

Table 4-17 - Estimation of First Order Exponential Decay Rates from First-Order Decay Model

Condition	Soil O.C.	K_D (hr^{-1})	R^2
High Buffer – High Temperature	0.50%	0.064	98.43
	0.30%	0.125	90.99
	0.04%	0.092	99.97
Medium Buffer – High Temperature	0.50%	0.06	99.21
	0.30%	0.055	96.25
	0.04%	0.033	81.65
Low Buffer – High Temperature	0.50%	0.093	79.92
	0.30%	0.126	N/A
	0.04%	0.073	83.26
High Buffer – Low Temperature	0.50%	0.027	90.28
	0.30%	0.067	92.83
	0.04%	0.03	80.83
Medium Buffer – Low Temperature	0.50%	0.131	N/A
	0.30%	0.035	91.7
	0.04%	0.09	N/A
Low Buffer – Low Temperature	0.50%	0.059	76.92
	0.30%	0.027	96.99
	0.04%	0.037	88.56

Table 4-16 shows that the highest growth rate occurs during the summer and with high organic fraction soil (0.5%) as opposed to the lowest growth rate which also occurs for the same 0.5% organic fraction soil but during the winter. As indicated from Table 4-17, the decay rates generally tend to decrease during the winter than those estimated for the summer, except in the case of applying a medium concentration buffer. For the latter, the values in Table 4-17 suggest that the decay rate in the winter are higher than they are in the summer.

Figure 4-28 as well as **Figure 4-29** show that in many cases, it can be assumed that there is a direct relationship between the growth coefficient of the microbial population in the soil and the organic fraction of the soil. The figures show that this relationship can be represented by a zero-order relationship, expressed in the equation ($K_G = A \times F_{o.c.}$) where F_{oc} is the organic fraction of the soil, and A is the slope. The R^2 value indicated that, at least for those two particular cases, a strong linear relationship between K_G and the soil organic fraction.

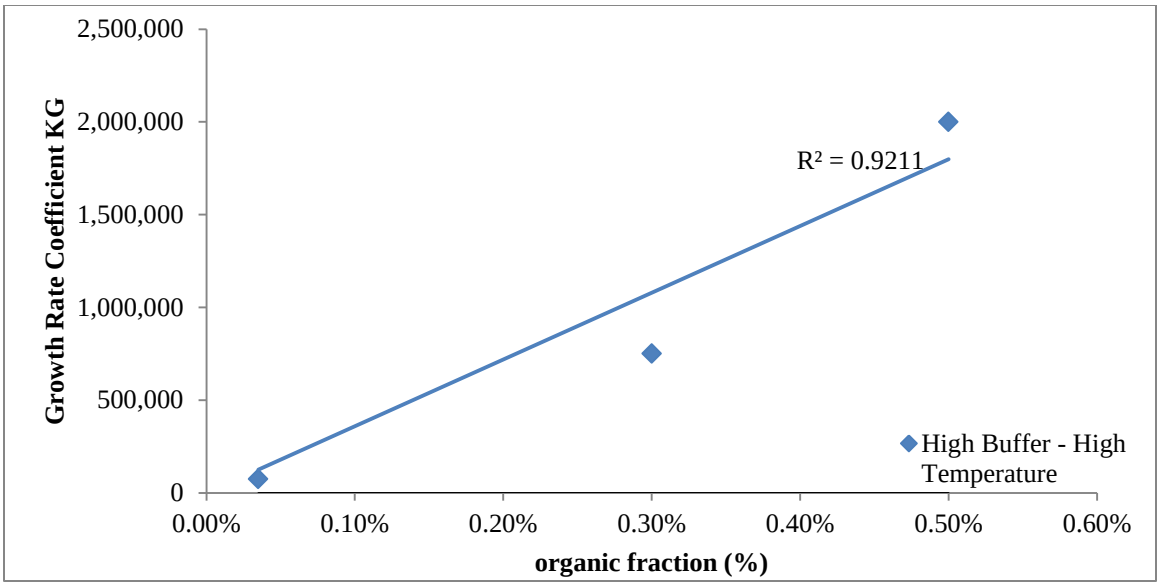

Figure 4-28- Growth Rate Coefficient vs. Organic Fraction for the Case of High Buffer Concentration at High Temperature

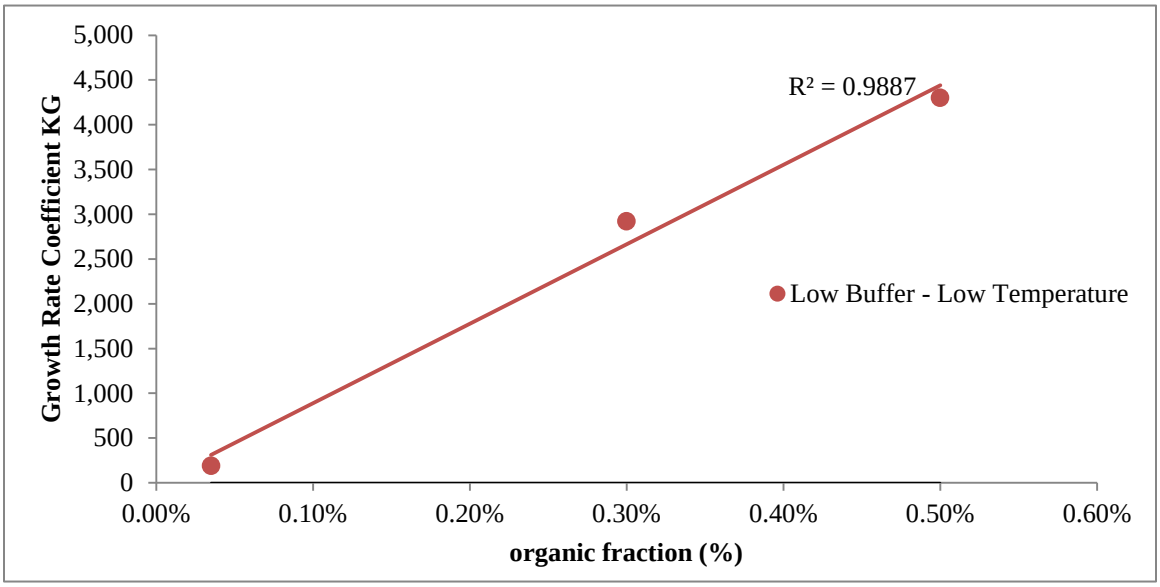

Figure 4-29 - Growth Rate Coefficient vs. Organic Fraction for the Case of Low Buffer Concentration at Low Temperature

In several cases a lag phase was observed before growth, such as in the case of the bacteria survival experiment using a medium concentration buffer (3,600 CFU/100 ml) with the soil "as is" and at low temperature, which typically follows the theoretical life cycle of the bacteria. The Monod model was therefore tested for this particular case and it seemed to fit well with the lag phase and the growth of the bacteria, and shown in **Figure 4-30**. However, when using "typical" values of Monod constants, the Monod model could not simulate the rapid rate of decay observed in many of these experiments (especially the summer experiments).

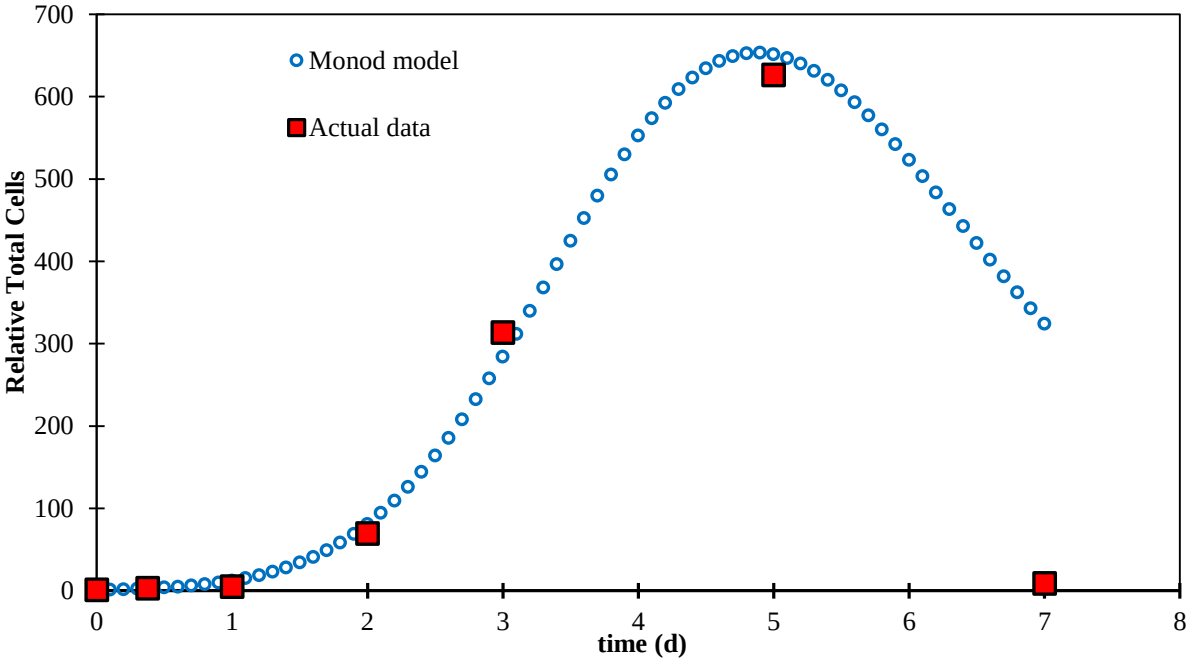

Figure 4-30 - Testing the Monod Model Using Data from Bacteria Survival Experiment with "As Is" Soil, Medium Concentration Buffer (3,600 CFU/100 ml), and at Low Temperature

5 Conclusion and Recommendations

More widespread reuse of treated wastewater in agriculture will clearly improve the water resources profile of countries in the MENA region. However, the practice must be safe and sustainable if it is truly to be to advantage; otherwise the contamination risks to human health and the environment will outweigh the gains. Moreover, effective guidelines for reuse of treated wastewater must account for local, site-specific variables – most notably soil and climate characteristics – if they are to be practical and effective. This research was an attempt to address these concerns by assessing the growth and survival of an indicator biological contaminant, *E. coli*, in static soil columns using a local desert soil under ambient conditions during different seasons. Analysis of the depth-concentration profiles deriving from these experiments resulted in the following major conclusions.

- The survival profiles observed in this study showed that the general trend for the bacteria life cycle in soil involves growth up to a peak value followed by decay. Depending on conditions of soil organic content, ambient temperature, and initial *E. coli* concentration, peak cell values were as much as three orders of magnitude higher than the number of viable cells initially added to a "clean" soil column. The presence of sunlight and the seasonal temperature are major factors affecting the survival of the bacteria in the soil since they also alter the soil properties that impact the bacterial growth and survival as a function of depth; *e.g.*, the soil moisture content.
- In most cases for the summer experiments, *E. coli* growth peaked at ~24 hours, after which they start dying off. In most cases, virtually all bacteria were inactivated after 5-7 days from the addition of the *E. coli* buffer solution. The most rapid decay occurred in the summer versus winter, especially when the bacteria solution introduced to the soil is of concentration higher than or equal to 30,000 CFU/100 ml and lower than or equal to 750 CFU/100 ml. At medium concentration (~ 5000 CFU/100 ml), however, the opposite was noticed and the specific reasons for that is unclear.
- At lower temperature, the peak value of the bacterial growth most often occurred at a longer time frame of 72 to even 96 hours, and usually this growth was prolonged with associated slower rate of decay.
- The increase of organic content of the test soil using uncomposted manure generally, as expected, resulted in increased peak *E. coli* biomass growth in soil columns. The organic content also was responsible for the prolonged survival of bacteria in the soil. However, it must be kept in mind that these effects on both the extent and rates of growth and decay were also influenced by the temperature at the time of irrigation, as well as the initial concentration of the bacterial solution.
- The bacterial concentrations were also observed to change with depth in the soil column throughout the duration of an experiment. At high temperatures, such as in the summer, the *E. coli* cells were

mostly found at deeper depths where there is also higher residual water content since these layers dry more slowly than the surface. The more intense solar radiation in summer is also more effective at inactivating coliform bacteria near the surface.

- Some of the above trends were quantified by estimating *E. coli* growth using a zero-order growth model assumption, whereas the decay rate was assumed to follow first-order exponential decay.

The results of this study should promote guidelines for reuse of treated wastewater in agriculture that are more consistent with local site-specific variables. For instance, the growth peaks and survival pattern of *E. coli* in the desert test soil could lead to modification of irrigation and work cycles so that field workers can refrain from contact with the topsoil during peak concentrations until natural die-off has occurred. Specifically, field workers should avoid handling irrigated soil entirely for 2-3 days after irrigation during summer and between 4-5 days during winter, but this can be estimated depending on the initial microbial concentration of the water used. In addition, the growth and decay coefficients for the varying conditions can be used in mass transport models to calculate quantitative risks to both human health (*e.g.*, field workers) and to the environment (*e.g*., risk of ground water contamination).

Further research that naturally proceeds from these studies includes the following.

- The methods developed in this work should be used to determine the effects of other constituents in typical treated wastewaters on the soil-bacteria interactions identified in this work. In particular, the impacts of ionic strength (total dissolved solids), total organic carbon, nutrients, and common mineral salts that are likely present in treated wastewaters on the growth and decay of bacteria in soil are important to identify and account for.
- The same study can also be conducted on other types of sandy and slightly clayey soils that are commonly used in agriculture in Egypt and the MENA region. A more careful analysis of soil moisture versus depth in these studies would also be helpful.
- Flow-through soil column studies at various loading rates with both synthetic and actual treated wastewater should be conducted in order to obtain data for calibration of mass transport modeling using bacteria growth and rate coefficients obtained from this work. A verified model can then be used to predict bacterial concentrations in time and space for calculation of risks in actual field applications of treated wastewater in irrigation.

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Appendix 1:
International and American Guidelines for the Reuse of Wastewater in Agriculture

WHO Guidelines (1989)

Category	Reuse condition	Exposed group	Intestinal nematodes[b] (arithmetic mean no. of eggs per litre[c]	Fecal coliforms (geometric mean no. per 100 ml[c])	Wastewater treatment expected to achieve the required microbiological quality
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parksd[d]	Workers, consumers, public	1	1000[d]	A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment
B	Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees[e]	Workers	1	No standard recommended	Retention in stabilization ponds for 8-10 days or equivalent helminthes and fecal coliform removal
C	Localized irrigation of crops in category B if exposure of workers and the public does not occur	None	Not applicable	Not applicable	Pretreatment as required by the irrigation technology, but not less than primary sedimentation

[a] In specific cases, local epidemiological, socio-cultural and environmental factors should be taken into account, and the guidelines modified accordingly.

[b] *Ascaris* and *Trichuris* species and hookworms.

[c] During the irrigation period.

[d] A more stringent guideline (<200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.

[E] In the case of fruit trees, irrigation should cease two weeks before fruit is picked, and no fruit should be picked off the ground. Sprinkler irrigation should not be used.

Revised WHO Guidelines (2000)

Category	Reuse condition	Exposed group	Irrigation techniques	Intestinal nematodes[b] (arithmetic mean no. of eggs per litre[c]	Faecal coliforms (geometric mean no. per 100 ml[d])	Wastewater treatment expected to achieve the required microbiological quality
A (unrestricted)	A1 for vegetable and salad crops, eaten uncooked, sports fields, public parksd[e]	Workers, consumers, public	Any	≤ 0.1[F]	$\leq 10^3$	Well-designed series of waste stabilization ponds (WSP), sequential batch-fed wastewater storage and treatment reservoirs (WSTR) or equivalent treatment (*e.g.*, conventional secondary treatment supplemented by either polishing ponds or filtration and disinfection)
B	cereal crops, industrial crops, fodder crops, pasture and trees[g]	B1 Workers (but no children < 15 years)nearby communities	Spray or sprinkle	≤ 1	$\leq 10^5$	Retention in WSP series including one maturation pond or in sequential WSTR or equivalent treatment (*e.g.*, conventional secondary treatment supplemented by either polishing ponds or filtration)
		B2 or B1	Flood/furrow	≤ 1	$\leq 10^3$	As for category A
		B3 workers including children <15 years, nearby communities	Any	≤ 0.1	$\leq 10^3$	As for category A
C	Localized irrigation of crops in category B if exposure of workers and the public does not occur	None	Trickle, drip or bubbler	Not applicable	Not applicable	Pretreatment as required by the irrigation technology, but not less than primary sedimentation

[a] In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account and the guidelines modified accordingly.
[b] Ascarisand Trichurisspecies and hookworms; the guideline limit is also intended to protect against risks from parasitic protozoa.
[c] During the irrigation season (if the wastewater is treated in WSP or WSTR which have been designed to achieve these egg numbers, then routine effluent quality monitoring is not required).
[d] During the irrigation season (faecal coliform counts should preferably be done weekly, but at least monthly).
[E] A more stringent guideline limit (4200 faecal coliforms/100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.
[F] This guideline limit can be increased to41 egg/l if (i) conditions are hot and dry and surface irrigation is not used or (ii) if wastewater treatment is supplemented with anthelmintic chemotherapy campaigns in areas of wastewater reuse.
[g] In the case of fruit trees, irrigation should stop two weeks before fruit is picked, and no fruit should be picked off the ground. Spray/sprinkler irrigation should not be used.

US Guidelines for Some States

The following regulations were presented by a US Environmental Protection Agency (US EPA) report for some states relating to the required treatment for the reuse of wastewater in different practices of agriculture (U.S. Environmental Protection Agency (US EPA), 2004), which is reasonable because different applications require different allowable limits of contamination depending on the extent of exposure.

Regulations in Some States in the US for Reuse of Wastewater in Food Crops Cultivation

	Arizona	**California**	**Florida**	**Hawaii**	**Nevada**	**Texas**	**Washington**
Treatment	Secondary Treatment, filtration, and disinfection	Oxidized, coagulated, filtered, and disinfected	Secondary Treatment, filtration, and high- level disinfection	Oxidized, filtered, and disinfected	Secondary Treatment and disinfection	NS (1)	Oxidized, coagulated, filtered, and disinfected
BOD5	NS	NS	20 mg/l $CBOD_5$	NS	30 mg/l	NS	30 mg/l
TSS	NS	NS	5 mg/l	NS	NS	NS	30 mg/l
Turbidity	2 NTU (Avg.)	2 NTU (Avg.)	NS	2 NTU (Max)	NS	3 NTU (Max)	2 NTU (Avg.)
	5 NTU (Max)	5 NTU (Max)					5 NTU (Max)
Coliform	**Fecal**	**Total**	**Fecal**	**Fecal**	**Fecal**	**Fecal**	**Total**
	None Detectable (Avg.)	2.2/100 ml (Avg.)	75% of samples below detection	2.2/100 ml (Avg.)	200/100 ml (Avg.)	20/100 ml (Avg.)	2.2/100 ml (Avg.)
	23/100 ml (Max.)	23/100 ml (Max. in 30 days)	25/100 ml (Max.)	23/100 ml (Max. in 30 days)	400/100 ml (Max.)	75/100 ml (Max.)	23/100 ml (Max.)
(1) NS - Not Specified By State Regulations							

Regulations in Some States in the US for Reuse of Wastewater in Non-Food Crops Cultivation

	Arizona	**California**	**Florida**	**Hawaii**	**Nevada**	**Texas**	**Washington**
Treatment	Secondary Treatment and disinfection	Secondary-23, Oxidized, and disinfected	Secondary Treatment, and basic disinfection	Oxidized, filtered, and disinfected	Secondary Treatment and disinfection	NS (1)	Oxidized and disinfected
BOD5	NS	NS	20 mg/l $CBOD_5$	NS	30 mg/l	5 mg/l	30 mg/l
TSS	NS	NS	20 mg/l	NS	NS	NS	30 mg/l
Turbidity	NS	NS	NS	2 NTU (Max)	NS	3 NTU (Max)	2 NTU (Avg.)
							5 NTU (Max)
Coliform	**Fecal**	**Total**	**Fecal**	**Fecal**	**Fecal**	**Fecal**	**Total**
	200/100 ml (Avg.)	23/100 ml (Avg.)	200/100 ml (Avg.)	2.2/100 ml (Avg.)	200/100 ml (Avg.)	20/100 ml (Avg.)	23/100 ml (Avg.)
	800/100 ml (Max.)	240/100 ml (Max. in 30 days)	800/100 ml (Max.)	23/100 ml (Max. in 30 days)	400/100 ml (Max.)	75/100 ml (Max.)	240/100 ml (Max.)
(1) NS - Not Specified By State Regulations							

Regulations in Some States in the US for Reuse of Wastewater in Unrestricted Recreational Facilities

	Arizona	California	Florida	Hawaii	Nevada	Texas	Washington
Treatment	NR (1)	Oxidized, coagulated, filtered, and disinfected	NR	NR	Secondary treatment and disinfection	NS (2)	Oxidized, coagulated, filtered, and disinfected
BOD5	NR	NS	NR	NR	30 mg/l	5 mg/l	30 mg/l
TSS	NR	NS	NR	NR	NS	NS	30 mg/l
Turbidity	NR	2 NTU (Avg.) 5 NTU (Max)	NR	NR	NS	3 NTU (Max)	2 NTU (Avg.) 5 NTU (Max)
Coliform	**Fecal**	**Total**	**Fecal**	**Fecal**	**Fecal**	**Fecal**	**Total**
	NR	2.2/100 ml (Avg.) 23/100 ml (Max. in 30 days)	NR	NR	2.2/100 ml (Avg.) 23/100 ml (Max. in 30 days)	20/100 ml (Avg.) 75/100 ml (Max.)	2.2/100 ml (Avg.) 23/100 ml (Max.)
(1) NR - Not Regulated by the State (2) NS - Not Specified by State Regulations							

Regulations in Some States in the US for Reuse of Wastewater in Restricted Recreational Facilities

	Arizona	California	Florida	Hawaii	Nevada	Texas	Washington
Treatment	Secondary Treatment, filtration, and disinfection	Secondary-23, Oxidized, and disinfected	NR(1)	Oxidized, filtered, and disinfected	Secondary treatment and disinfection	NS (2)	Oxidized and disinfected
BOD5	NS	NS	NR	NS	30 mg/l	20 mg/l	30 mg/l
TSS	NS	NS	NR	NR	NS	NS	30 mg/l
Turbidity	2 NTU (Avg.) 5 NTU (Max)	NS	NR	2 NTU (Max)	NS	NS	2 NTU (Avg.) 5 NTU (Max)
Coliform	**Fecal**	**Total**	**Fecal**	**Fecal**	**Fecal**	**Fecal**	**Total**
	None Detectable (Avg.) 23/100 ml (Max.)	2.2/100 ml (Avg.) 23/100 ml (Max. in 30 days)	NR	2.2/100 ml (Avg.) 23/100 ml (Max. in 30 days)	200 /100 ml (Avg.) 23/100 ml (Max. in 30 days)	200 /100 ml (Avg.) 800 /100 ml (Max.)	2.2/100 ml (Avg.) 23/100 ml (Max.)
(1) NR - Not Regulated by the State (2) NS - Not Specified by State Regulations							