REDUCTION OF PATHOGENS IN BIOSOLIDS IN MEXICO USING SOLAR DRYING BEDS

by

Teódulo Domínguez Sánchez

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SIGNED: Teódulo Domínguez Sánchez

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

_____ 10/17/2005

Dr. Christopher Y. Choi Date
Associate Professor
Department of Agricultural & Biosystems Engineering

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TABLE OF CONTENTS

TABLE OF CONTENTS- *Continued*

LIST OF FIGURES

LIST OF FIGURES- *Continued*

LIST OF TABLES

LIST OF TABLES- *Continued*

ABSTRACT

In this study, die-off patterns of helminth ova, fecal coliforms, and *Salmonella spp*. in biosolids were documented using three small-scale sand drying beds located in a greenhouse. Treatments involved tilling the biosolids with differing frequencies. The results indicate that the inactivation rate for helminth ova was 0.88, 0.55, and 0.22 eggs/4 g TS day^{-1} for the intensively-tilled, moderately-tilled, and control beds, respectively. Achievement of Class A criteria was only possible in the intensively-tilled bed by Day 70 of the experiment. *Salmonella* spp. were inactivated to Class A levels in 9 days for the intensively and moderately-tilled beds. Regrowth of *Salmonella* spp. occurred thereafter in all beds, but high levels were seen only in the control bed. Fecal coliforms reached Class A criteria late in the experiment. Tilling treatments enhanced the inactivation rate of helminth ova and offer a potentially cost-effective method of pathogen reduction.

CHAPTER 1 – INTRODUCTION

1.1 Problem Definition and Objective

The quality of wastewater treatment and the fate of sewer sludge in Mexico have both become important issues recently. In 1994, the National Water Commission of Mexico reported that 22% of Mexico's total wastewater was treated (Cabezut and Sanchez, 1999). As of 2002, the Commission reported that 26% of municipal and 15.3% of industrial wastewater in Mexico were subject to treatment (National Water Commission Publication, 2004).

Mexico currently irrigates 260,000 ha of agricultural land with 190 m^3/s of wastewater generated in 55 cities (Jimenez, 2005). Approximately 374 m^3/s of wastewater is generated in Mexico; Mexico City alone generates as high as 80 m^3/s during the rainy season (Jimenez and Landa, 1998). Raw sewer water from Mexico City is mainly sent to the Mezquital Valley and used to irrigate an area of 90,000 ha (see Figure 1.1). Despite the many benefits of the various nutrients in this water, the incidence of illness is 13 times higher in areas which rely on untreated wastewater for irrigation than in areas which use treated wastewater (Jimenez and Landa, 1998). Thus, untreated wastewater has been identified as a major health concern, and it is important to build additional wastewater treatment facilities in the near future.

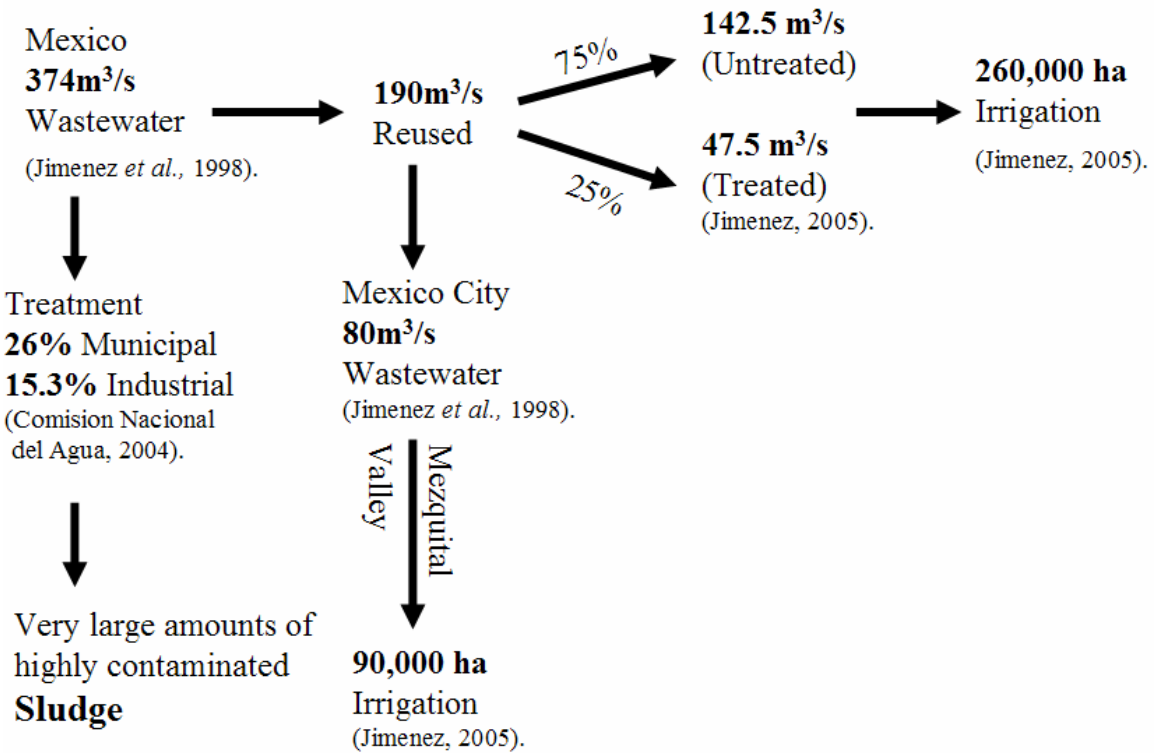

Figure 1.1. Overview of wastewater treatment and reuse quantities in Mexico

Increased mechanical treatment of sewer water will in turn require Mexico to address large-scale operations for the disposal and recycling of solid residuals, which are the byproducts of wastewater treatment. Currently, existing wastewater treatment facilities already generate large amounts of solid residuals annually. However, the Mexican government has yet to develop adequate treatment and recycling strategies for sewer sludge.

The disposal and recycling of sewer residuals can be a significant threat to public health and the environment if pollutant levels are not reduced or the land application of

biosolids is improperly managed. In the United States, pollutant categories and their detrimental levels are defined in the Biosolids Rule (EPA, 1994) as heavy metals, pathogens, and macronutrients (i.e., nitrogen and phosphorus). In some areas of Mexico, sewer sludge has generally been dried or composted for beneficial reuse (UNEP, 2000). However, systematic research efforts to disinfect the sewer sludge are lacking, and corresponding national policies and strategies for land application have yet to be developed and implemented.

Treated sewer sludge is termed “biosolids.” In order to recycle its biosolids and avoid further environmental contamination and health risk issues, it is important that Mexico investigate the heavy metal, macronutrient, and microbial characteristics of generated biosolids and develop guidelines for their production and reuse. One economical method of disinfecting biosolids is to dry the sludge on solar drying beds. Drying beds are common in dewatering sewer sludge in the United States (Metcalf and Eddy, 2004), and these are also being utilized to disinfect biosolids (Choi *et al.*, 2005). The disinfection of biosolids in open solar drying beds is highly dependent on climatic factors such as air temperature, wind, relative humidity, and rainfall. Therefore, it is prudent to study the pattern of inactivation of pathogenic microorganisms within the relevant climatic zone and develop guidelines specific to the climatic zone prior to integrating the biosolids into the environment.

In the United States, pathogens and pathogen indicators are utilized to establish designated microbial levels and define corresponding restrictions for the land application of biosolids. The classification of biosolids and various treatment methods are also listed, including the maximum detection levels for the various pathogens: enteric viruses, bacteria (*Salmonella* spp.), parasites (helminth ova), and pathogenic indicators (fecal coliforms), as shown in Table 1.1.

Table 1.1. Pathogen Density Levels (40 CFR Part 503) (USEPA, 1994)

Classification of Biosolids	Pathogen or Indicator Criteria (Dry weight)
Class A	**Fecal Coliforms** < 1000 MPN/g Total Solids or ***Salmonella*** < 3MPN/4g Total Solids and **Enteric Viruses** < 1 PFU/4g Total Solids and **Helminth Ova** < 1/4g Total Solids
Class B	**Fecal Coliforms** < 2×10^6 MPN/g Total Solids

In recent years, scientists in Mexico have made progress towards characterizing the heavy metal and pathogen concentrations in the biosolids which are being produced. Also, Mexico is currently investigating the best methods to address the reduction of pathogen levels in its biosolids. Certainly, the regulations currently practiced in countries like the United States and Europe should be taken into consideration for the development of national guidelines and best management practices for the proper disposal and reuse of biosolids in Mexico. However, it is equally important that the indigenous influent levels

of fecal coliforms, *Salmonella* spp., and helminth ova which exist in waste residuals in Mexico be taken into account when developing specific treatment methods. For example, in the United States, helminth ova concentrations are typically very low, approximately 1 egg/g TS (Total Solids) (Barrios *et al.*, 2004), while in Mexico, maximum influent levels have approached 657 eggs/g TS (Nelson *et al.*, 2003).

Previous experiments at the Green Valley WWTP (Wastewater Treatment Plant) in Pima County, Arizona and in Quartzsite, La Paz County, Arizona have shown that Class A biosolids can be achieved using open solar drying beds (O'Shaughnessy *et al.*, 2005). High daily average temperatures and rapid evaporation rates at these sites enhanced the destruction of pathogenic microorganisms. Due to the high average daily temperatures and low relative humidity at Quartzsite, Arizona, Class A biosolids are obtained in about two (2) weeks during the summer season in terms of fecal coliform criteria and within one (1) week using *Salmonella* spp. criteria (Choi *et al.*, 2005). This process is shortened by incorporating tilling treatments, which rapidly reduce the moisture content of the biosolids. In Green Valley, Arizona, it typically takes 28 days to achieve Class A criteria using fecal coliform criteria with no tilling and only 14 days when the biosolids are tilled two (2) times per week during the summer season. Five (5) days were required to reduce *Salmonella* spp. levels to Class A criteria. The average daily summer temperatures and relative humidity levels at Quartzsite were 32.0°C and 12.6%, respectively. In Green Valley, the average temperature and relative humidity levels were 32.4°C and 21.7%,

respectively. The influent levels for helminth ova at both locations were determined to be zero (0) organisms/4g dry weight of biosolids at the beginning of each experiment.

In areas with lower daily average temperatures, higher relative humidity, and frequent rainfall (such as Cuernavaca, Morelos, Mexico), a time lag for achieving Class A biosolids is expected. Furthermore, the high helminth ova concentrations generally found in Mexico's sludge will require higher temperature-time regimes as well as increased desiccation rates in comparison to those found in arid zones in Arizona. The ambient air temperature inside of a controlled environment can be raised and the drying beds relatively sheltered from rainfall to prevent regrowth. It is also expected that helminth ova can be destroyed more efficiently in a controlled environmental system by increasing the number of heat units impacting the biosolids. According to a recent study, either high temperatures (of up to 57°C) or high pH levels (approaching 12) are effective treatments leading to the destruction of helminth ova (Jimenez *et al.,* 2001). Tilling paired with a controlled environment can supplement the rate of desiccation and improve the inactivation rate of pathogens in biosolids in a wet and humid climate.

The primary objective of this experiment was to determine the rate of inactivation of helminth ova, fecal coliforms, and *Salmonella* spp. in sludge after applying tilling treatments over time in a semi-controlled environment. Real-time data measurement and acquisition were used to monitor the key environmental factors within the semi-

controlled environment, such as air temperature, relative humidity, solar radiation, and the temperature and moisture of the biosolids in the drying beds.

Due to the high number of helminth ova found in biosolids in Mexico and the persistency of this parasite, the focus of this research was the inactivation or destruction of helminth ova. In Mexico, about 33% of the population is infected with *Ascaris*, the most predominant helminth genera found in sludge and also one of the most resistant to destruction (Jimenez *et al.,* 2000). Helminth ova are not required to be evaluated under the current Mexican regulations governing the production of biosolids; however, their pervasive presence, high concentrations in sewer sludge, and their adverse impact on public health requires that special attention be devoted to finding an efficient and economically feasible method towards their reduction.

CHAPTER 2 - LITERATURE REVIEW

2.1 The Status of Biosolids in Mexico

Land application of biosolids represents a great opportunity for use as a fertilizer in arid and semi-arid regions of the country, which represents 75% of the total land in Mexico (Jimenez and Chavez, 1997). Prior to 1997, there were no full-scale biosolids utilization systems in Mexico, and most of the untreated municipal sludge was disposed of in watercourses or stored in storage areas or uncontrolled landfill sites. Currently, a limited amount of biosolids are being land-applied in agricultural fields with no regulations regarding their quality or guidelines for land application.

Most of the wastewater treatment facilities constructed in Mexico are waste stabilization ponds (WSP), which are comprised of serial operating ponds and where treatment of wastewater consists of removing organic matter and solids by sedimentation or biological and chemical processes (Nelson *et al.*, 2003). This treatment results in many operational problems because sludge management is not incorporated into the design process. This could be attributed to a lack of available information on accumulation rates, sludge distribution within ponds, and chemical and biological characteristics (Nelson *et al.*, 2003). More than 400 stabilization ponds have been built since 1980. In 1998, the Mexican government developed a program to construct additional facilities within the country in southern, central, and northern Mexico (Escalante *et al*., 2000).

In developed countries like the United States, the quality of biosolids is heavily regulated; municipalities and industries must meet the requirements established by the US Environmental Protection Agency (EPA) to incorporate biosolids into agricultural fields or to distribute/sell the material as a soil amendment or fertilizer. In Mexico, on the other hand, treated solid residuals are dumped into water bodies after complying only with BOD (Biological Oxygen Demand) limits; microbiological qualifications established by national standards are either not enforced or largely disregarded (Escalante *et al*., 2000). In the Texcoco WWTP (Wastewater Treatment Plant), biosolids are dumped onto the land within the confinements of the WWTP with no system for further treatment or recovery of the solid residuals for recycling (site visit to Texcoco WWTP, May 2005).

By 1998, Ciudad Juarez, Mexico started using biosolids when local authorities realized they needed to focus on biosolids as a major component of the city's water recycling program. The UNU-INWEH (United Nations University International Network on Water, Environment, and Health) coordinated this project and has established similar biosolids projects in the Mexican states of Puebla, Querétaro, and Coahuila as part of a National Biosolids Management Program proposed by the National Water Commission of Mexico (CNA) (UNEP, 2000). Generally, biosolids have been dried or composted for beneficial reuse.

In the Mexican State city of Texcoco, composting of waste products using earthworms (vermicomposting or vermiculture) has recently been utilized as one way for farmers to replace fertilizers. Approximately 40 companies are operating vermicomposting plants in

thirteen Mexican states (Sherman, 2002). This widespread use shows the importance of final residuals from wastewater treatment plants. However, socio-economic, cultural, political, and interpersonal issues still prevent the large-scale treatment and use of such biosolids.

Although application of biosolids has multiple benefits, high concentrations of pathogens and parasites can threaten public health. In Mexico City, sludge contains very high pathogen concentrations, up to 150 helminth ova per gram of total solids (Jimenez and Chavez, 1997). Thus, infectious diseases could be caused by parasitic microorganisms found in residual sludge. In Mexico, helminthiasis affects children exposed to untreated water 16 times more than children exposed to treated water (Jimenez, 2005). Despite the fact that wastewater generally has lower levels of helminth eggs than sludge, the potential negative impact of wastewater reuse on public health is evident. The reduction of pathogens and parasites to acceptable levels is an important and challenging task that ultimately depends on the type of treatment, temperature, and other climatic conditions particular to the treatment site itself.

Beyond microbial quality concerns of biosolids, there also exists the concern regarding high levels of heavy metal concentrations. The Mexican city of Leon, Guanajuato, produces amounts of tannery sludge that are as high as 64,320 tons per year (Barajas and Dendooven, 2000). Disposal of this sludge to amended agricultural lands can incorporate great amounts of N and organic matter into eroded soil. However, high heavy metal concentrations may cause significant adverse impacts on the environment.

Textile industries generate 90% of the biosolids deposited into the Lerma River of Mexico State; those remaining are deposited by households. Wastewater in this area is treated by the Reciclagua wastewater treatment plant, which demands that companies reduce BOD levels to less than 1000mg/l, lipids to less than 150mg/l, and phenols to less than 1mg/l before incorporating their wastes into the treatment process (Franco *et al.,* 2002).

2.2 Sludge Characteristics

2.2.1 Physical Quality

A recent study in Central Mexico on sludge characteristics, accumulation rates, and uniformity through ponds shows a per capita sludge accumulation rate between 0.021 and 0.036 m^3/person/yr; the estimated recommended rate was 0.04 m^3/person/yr in both anaerobic and facultative ponds after confirming several past studies (Nelson *et al.,* 2003). In this study, sludge distribution varied through ponds. In anaerobic ponds with multiple inlets and shorter hydraulic retention times (HRT), sludge distribution was uniform, while in facultative ponds with single inlets and longer HRTs, sludge accumulation occurred mostly in front of the inlets. After deposition occurred, compaction and anaerobic degradation (both factors affecting sludge volume) were similar in all ponds. A regression equation relating TS concentration to the thickness of the sludge layer was developed to evaluate different processes in sludge removal. After one year, the rate of anaerobic degradation decreased significantly and the first-order inactivation rate continued to fluctuate constantly between 0.042 and 0.122 yr^{-1} in ponds (See Table 1.2). Average

concentrations of solids in sludge layer and accumulation rates are compared with those in the literature as cited by Nelson *et al.* (2003), in Table 1.3. In 1998, IMTA (Instituto Mexicano de Tecnología del Agua, or the Mexican Institute of Water Technology) analyzed influents and effluents in the waste stabilization ponds of La Reforma, Gomez Portugal, Guadalupe Victoria, González Ortega, and Chiapilla, using a software program, CREALE®, which was developed by the IMTA institute. The La Reforma Waste Stabilization Pond was found to have unsatisfactory results for its use in irrigation and its compliance with limits established on organic loads. Data is shown in Table 2.3.

To comply with national regulations, IMTA recommended the construction of another pond which would work as a maturation pond. To meet national regulation levels, the following guidelines should be met: BOD around 13mg/l, fecal coliforms around $6.8x10^2$ MPN/100ml, and no presence of helminth ova.

Table 1.2. Sludge characteristics for WSPs (Nelson *et al.*, 2003)

Pond Location	Type	Operation period (yr)	Mean TS (g/l)	Mean VS/FS	Accumulation rate		Sludge Total Vol. (%)	HRT without Sludge	HRT w/sludge	Sludge thickness Average (m)
					m^3/pers/yr	mm/yr				
Mexicaltzingo, Mexico State	Anaerobic	5	171	0.63	0.022	119	25.3	2.5	1.9	0.67
San Jose, Morelos	Facultative	6	NA	NA	0.036	21	8.2	24	22	0.15
Texcoco, Mexico State	Facultative	10	112	0.57	NA[a]	21	14.4	47	9.0	0.36
Xalostoc, Tlaxcala	Facultative	15	166	0.67	0.021	19	13.2	10.6	41	0.34

a: Unknown population NA: Not available VS: Volatile solids FS: Fixed solids HRT: Hydraulic residence time

Table 1.3. Sludge characteristics found in other studies (Nelson *et al.*, 2003)

Pond	Type	Operation period (yr)	Mean TS (g/l)	Mean VS/FS	Accumulation rate	
					m^3/pers/yr	mm/yr
Columbia	Anaerobic	2.6	NA	NA	0.055	NA
		5			0.040	
SE Brazil	Anaerobic	NA	172	0.62	0.023	77
				NA	0.026	53
NE Brazil	Anaerobic	2.5	39	1.5	NA	NA
France	Facultative	10	187	NA	NA	NA
France	Facultative	3-10	54-136	0.29-0.94	0.12	15-85
Mississippi, USA	Facultative	0.5-7	35-192	0.11-0.59	NA	15-51
Utah, USA	Facultative	7	59	2.23	NA	6.87
		13	77	4		8.1

NA: Not available

Table 1.4. Influent - effluent concentrations in La Reforma WSP (Escalante *et al.*, 2000)

Parameter	Influent	Effluent	Final effluent
BOD(mg/l)	101	50	20.2
TS(mg/l)	22,084	22,676	23,032
TSS(mg/l)	192	150	113.3
TDS(mg/l)	21,892	22,526	22,918
Fecal coliforms(MPN/100ml)	$42x10^5$	$91x10^3$	3,286

The situation at Gomez Portugal WSP showed that the operational flow was much less than the design flow. However, the results were similar to La Reforma WSP in that the effluent did not meet fecal coliform and helminth egg limits. To solve this problem, a facility upgrade was recommended, as observed in Table 1.5. The improvement of the effluent BOD was >50% and the number of fecal coliforms was reduced by 5 logs as compared to the existing treatment systems. The change in density levels of helminth ova was not reported. In a series of three ponds at the Guadalupe Victoria WSP, the first pond was found to be completely full, with a sludge depth of 1.5m and a water level of 10cm. It was also found that farmers frequently used the water before it could reach the third pond. The system was also designed only for organic load removal, see Table 1.6

Table 1.5. Analysis of several alternatives to the Gomez Portugal WSP (Escalante *et al.*, 2000)

Variables	Actual condition Case 1	Actual condition and design flow Case 2	Upgrade Case 1	Upgrade Case 2
Treatment train	BF	BF	A+F+5M	A+F+5M
Flow (l/s)	1.61	18.5	1.61	18.5
HRT (days)	945	19.42	43.96	43.96
Area (ha)	1.67	1.67	0.397	3.93
Effluent BOD (mg/l)	77.99	87.3	20.72	20.72
Remotion (%)	73.2	70	92.88	92.88
Effluent Fecal Coliforms PN/100ml)	99,390	4.2×10^{6}	961	961
Remotion (%)	99.98	94.50	99.99	99.99
Influent BOD (mg/l)	291			
Influent Fecal Coliforms PN/100ml)	8.32×10^{7}			

A: Anaerobic, BF: Baffled facultative, F: Facultative, M: Maturation.

Table 1.6. Design for organic load removal (Escalante *et al.*, 2000)

Variables	Actual flow	Design flow
BOD (mg/l)	316	31.45
Fecal coliforms (MPN/100ml)	2.7×10^{7}	27,240
Flow rate (l/s)	70.2	35
HRT (days)	2.9	25.73
Surface load (kg/ha-day)	1,729.4	

BOD: Biological oxygen demand, HRT: Hydraulic residence time

The HRT is lower than recommended (5-35 days) and the surface load is larger than recommended (350 kg/ha-day). An analysis of the design flow shows that it is not possible to comply with the microbiological standards. Thus, expansion of the wastewater plant by introducing an anaerobic pond followed by a facultative and four maturation ponds with a HRT of 37.7 days would be needed to achieve the minimum qualifications. These systems, including the Chiapilla and Gonzalez Ortega WSPs, were designed with only organic load criteria in mind; they do not account for hydraulic load capabilities. These systems resulted in low quality wastewater. Effluents, then, did not comply with local regulations for discharge into streams or use in agriculture. In addition, several issues were detected; there were often hydraulic problems because of bad inlet and outlet locations, influent and effluent qualities were not monitored periodically, and helminth eggs were not monitored at all (Escalante *et al.*, 2000). If many more WSPs are to be constructed in the future, those design and operational issues which have already been detected should be taken into consideration in order to solve these problems.

2.2.2 Microbial Quality - Helminth Eggs, Fecal Coliforms, and *Salmonella* spp. in Sludge

The population of Mexico has reached 100 million people (INEGI, 2000), and because of the highly concentrated population in this area, wastewater effluent and biosolids are produced in very large quantities that makes them difficult to handle in a proper manner. As a result, these materials are inadequately treated to protect human health and the environment and are also being disposed of improperly. Wastewater effluent and solid residuals from wastewater treatment plants contain a number of enteric pathogens,

viruses, bacteria, and parasites that can cause diseases such as stomach illness, vomiting, diarrhea, and death, particularly in the cases of the very young and the immunocompromised. Table 1.7 shows helminth eggs and fecal coliform values in a study conducted in three primary stabilization ponds in Central Mexico (Nelson *et al.*, 2003). Helminth eggs seemed to increase over time in the stabilization pond of Mexicaltzingo, decrease in the stabilization pond of Texcoco, and remain constant in the stabilization pond of Xalostoc. It was thought that helminth eggs at the stabilization pond of Texcoco could have been destroyed by industrial discharges from Mexico City and the high salinity of the soil in this area.

Table 1.7. Mean and maximum concentration of diferent microorganisms in the sludge layers (Nelson *et al.*, 2003)

Location	Total* helminth eggs, eggs/g TS		Viable helminth eggs, eggs/g TS		Somatic coliphage pfu/g TS		F+ coliphage, pfu/g TS		Fecal coliform bacteria, MPN/g TS		Fecal enterococci, pfu/g TS	
	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max
Mexicaltzingo	129	184	25	55	--	--	--	--	1.3×10^{5}	1.2×10^{7}	--	--
Texcoco	49	273	25	169	--	--	--	--	15.7×10^{4}	1.5×10^{7}	--	--
Xalostoc	277	657	48	257	5.3×10^{5}	4.2×10^{6}	1.2×10^{4}	1.3×10^{6}	3.1×10^{4}	4.4×10^{7}	3.4×10^{3}	7.9×10^{6}

*Most of the eggs found were *Ascaris* spp., as shown in Table 1.8.

Table 1.8. Species of helminth eggs present in sludge, in percentages of total eggs indicated above (Nelson *et al.*, 2003)

Pond	*Ascaris*	*Trichuris*	*Hymenolepis*	*Toxocara*
Mexicaltzingo	93.5	3.5	0.2	2.8
Texcoco	85.3	13	1.7	0.4
Xalostoc	87.4	2.2	9.8	0.6
Average	88.7	5.4	4.5	1.3

By the late 1980s, the Mexican Federal Government considered the Lerma River a highly-contaminated area because of the continuous discharge of untreated wastewater. After determining the fungi and pathogen concentrations, this wastewater was treated with different techniques to meet higher quality standards. Results obtained are shown in Table 1.9.

Table 1.9. Microorganisms present in biosolids and the maximum allowed limits (US EPA, 1994), (Franco *et al.*, 2002)

Pattern	Biosolids	Biosolids irradiated	Biosolids with Ca(OH)2	Biosolids heated at 60°C, 30 min	Minimum significant difference (P < 0.05)	US EPA (1994) maximum acceptable limits Class A	Class B
Fungi (CFU g^{-1} dry biosolids)	950[a]	1	43	48	242	NG	NG
Total coliforms (CFU g^{-1} dry biosolids)	$66x10^3$	1	NM	2100	13,594	NG	NG
Fecal coliforms (CFU g^{-1} dry biosolids)	1200	3	1000	1000	350	<1000	$<20x10^5$
Shigella spp. (CFU g^{-1} dry biosolids)	ND	ND	ND	ND	ND	NG	NG
Salmonella spp. (CFU g^{-1} dry biosolids)	250	1	ND	ND	30	<3	<300
Viable eggs of helminth (eggs kg^{-1} dry biosolids)	$30x10^3$	ND	ND	ND	ND	$<10x10^3$	$<35x10^3$

CFU: Colony forming units. a: Mean of four replicates. NG: Not given. NM: Not measured. ND: Not detectable.

Another study in a waste stabilization pond in Texcoco, Mexico characterized sludge which was generated in Mexico City. Samples were taken at different representative points and depths. The results showed that after 10 years of accumulating sludge, fecal coliforms and helminth eggs decreased as sludge layers grew deeper; however, significant concentrations were still present in even the oldest sludge layers. Fecal coliforms were 10^7 MPN/g TS at the top sludge layer and $3.7x10^3$ MPN/g TS at the deepest layer. Helminth egg numbers were 50eggs/L at the top layer and 14eggs/L at the deepest sludge layer (Nelson and Jimenez, 2000). Also see Table 1.10 below.

Table 1.10. Helminth egg concentrations as a function of depth in sludge cores (Nelson and Jimenez, 2000)

Estimated average sludge age (yrs)	Sample location in pond		Average
	Inlet	Outlet	
	(Viable eggs/g TS) /(Total eggs/g TS) (Percent viable)		
1.05	(169)/(273)(62)	(24)/(53)(46)	(50)/(102)(42)
3.36	(60)/(102)(59)	(2.9)/(7.7)(38)	(20)/(45)(44)
6.05	(46)/(58)(81)	(2.7)/(5.4)(50)	(15)/(23)(61)
8.75	(46)/(58)(79)	(2)/(2.8)(74)	(14)/(24)(68)

Fecal coliforms, helminth eggs, and *Salmonella* spp. reached levels greater than Class A criteria, based on the US EPA regulations, but could be classified as Class B. Class A biosolids can be applied without restrictions in the US, while Class B biosolids can be used for soil amendment, used as fertilizer on agricultural fields, and used for remediation of forest land and mine tailings (USEPA, 1994) with land-use restrictions. Pasteurization and $Ca(OH)_2$ applications reduced *Salmonella* spp. and helminth eggs;

however, the decrease in fecal coliforms did not result in Class A levels being met. A study recommended the storage of biosolids for at least three months while maintaining the pH well above 12 before application onto agricultural land. Pasteurization also reduced the helminth eggs, but a study by Barajas and Dendooven (2002) recommended twice-monthly pasteurization over a year to destroy *Ascaris* eggs (a particular strain of helminth) and other pathogens. Irradiation at 30 kGy significantly reduced all pathogens. This dose is much higher than values normally recommended for destroying nearly all *Ascaris* ova or fecal coliforms.

Sludge from an advanced municipal wastewater primary treatment facility in a neighboring town in Mexico City showed concentrations of 2.4×10^8 MPN/g TS, 2.3×10^6 MPN/g TS, and 105.2 eggs/g TS for fecal coliforms, *Salmonella* spp., and helminth ova, respectively (Barrios *et al.*, 2001). Allowing for anaerobic digestion of sludge, fecal coliforms decrease over time with up to 4 logs of magnitude and can reach Class B status. However, mesophilic anaerobic digestion does not reduce helminth ova. Another approach is acid treatment which has proved to reduce pH from 3.2 to 6.9 orders of magnitude for fecal coliforms and 69 to 90% efficiencies for helminth ova. Nevertheless, acetic acid presents a better performance with respect to microbial inactivation, cost, pH, and risk of application under laboratory studies. According to experiments, up to 15,000 ppm of acetic acid must be applied to inhibit bacterial regrowth, as lower doses promote acid tolerance.

Further studies showed that a combination of acetic acid and hydrogen peroxide, known as peracetic acid, was a better option than other processes already discussed (Barrios *et al.,* 2004). By applying 550 ppm of peracetic acid to fresh sludge, fecal coliforms were reduced by 6 logs with a contact time of 10 minutes; *Salmonella* was reduced to below detection limits with a contact time of 5 minutes and *Ascaris* eggs were reduced from an average of 102.5 to 15.8 eggs/g TS. In this study, application of peracetic acid presented advantages compared to other processes; e.g., shorter contact times (about 10 minutes) were needed to see high bacterial reductions. However, helminth eggs still show significant numbers, implying that high temperatures and pH are needed to inactivate these parasites.

In Mendez *et al.,* (2004), it is mentioned that ammonia has been successfully used as a disinfectant against bacteria in the form of NH_3 (non-ionized). Ammonia is not only effective against bacteria, but also against different genera of helminth ova in sludge. In a lab study, different concentrations of ammonia were applied to sludge with high initial pathogen concentrations to analyze an inactivation trend. Ammonia doses of 10–50% w/w of 20-30% v/v ammonium hydroxide (NH_4OH) solution were used at ambient temperature (20°C). Results showed that at a 20% dose, fecal coliforms were inactivated by 7 logs of and *Salmonella* spp. were inactivated by 5 logs which allowed for Class A achievement (Mendez *et al.,* 2004). But for helminth ova, a dosage of 50% ammonia was needed to inactivate 94% of viable helminth ova/g TS at 20°C. However, in this study, an important comparison was made between ammonia disinfection and lime stabilization. A

similar quicklime dosage 20% w/w (2 hrs contact times) achieved similar bacteria inactivation results but a lower effectiveness for helminth ova destruction. However, because the utilization of quicklime in sludge results in increasing salinity and increase in mass after treatment, ammonia is considered a better disinfectant.

2.2.3 Heavy Metal Quality

In some areas in Mexico and many other countries, heavy metal concentration is another concern when reusing biosolids. In the Lerma River of Mexico, high heavy nickel (Ni) and chromium (Cr) contamination levels have been found since the 1980s (Franco *et al.*, 2002).

In the state of Guanajuato, Mexico, tannery sludge presents high pathogen concentrations, toxic organic components, and also heavy metals, specifically Cr6+. Table 1.11 shows concentrations of heavy metals in sludge as compared to the upper limit concentrations given by the European Union for sewage sludge used in agriculture.

Table 1.11. Tannery sludge expressed in dry weight basis (Barajas and Dendooven, 2000).

Heavy metals	Concentration (mg/kg)	EU levels (mg/kg)
Zn	89	4000
Cu	81	1750
Ni	20	400
Cd	0.15	40
Cr	6690	---
Pb	35	1200

With the exception of Cr, concentrations of heavy metals are lower than those given by the European Union. However, it was previously found that 400 mg kg-1 of Cu and Cr, once incorporated into the soil, work adversely against microorganism activity at the cellular level by restraining biological activities; e.g., production of enzymes, urease, phosphatase, and dehydrogenase (Barajas and Dendooven, 2000). Plant growth is also inhibited by large concentrations of Cr. However, Cr found in tannery sludge was two times lower (414 mg Cr kg-1 soil) than the upper limit (900 mg kg-1 soil), and showed no effect on biological activities of microorganisms (Barajas and Dendooven, 2000). After treating wastewater with an advanced primary treatment (APT), the sludge produced was tested for heavy metals. The results are shown in Table 1.12 below. Maximum allowed limits were met according to the US EPA.

Table 1.12. Metal concentrations in biosolids (Jimenez *et al.*, 2000).

Parameter (mg/kg TS)	Experimental Data			US EPA Limits	
	Min	Max	Average	Max	Monthly Average
Arsenic	5	62	29	75	41
Cadmium	3	11	7	85	39
Copper	190	378	287	4300	1500
Mercury	0	44	8	57	17
Molybdenum	0	26	14	75	---
Nickel	26	208	102	420	420
Lead	58	112	75	840	300
Selenium	1.3	57	24	100	100
Zinc	415	1080	766	7500	2800

2.3 Techniques for Microbial Decay in Sludge

According to previous studies, the reduction of microbial concentrations in sludge can be achieved in the following ways:

- Liming is a good alternative (Jimenez *et al.*, 2001). However, serious odor problems result from the conversion of NH_4^+ to NH_3 gas. Yet, NH_3 increases the efficiency of bacteria destruction and helminth ova inactivation, its effect varies between open and closed systems.
- Applying irradiation with Co (30-KGy), and pasteurization are very effective. However, these are expensive methods, generally limited to research purposes only (Franco *et al.*, 2002).
- Acid use has also shown satisfactory results, particularly when using acetic acid mixed with hydrogen peroxide (peracetic acid).
- Ammonia was found to inactivate both bacteria and the most resistant parasites present in sludge. It further enhances biosolids as a soil fertilizer (with the additional benefit of carrying no risk of salinity problems) (Mendez *et al.*, 2004).

2.4 Helminth Ova Persistence and its Viability

Ascaris infection is a major concern around the world. Approximately one-third of the population is infected by intestinal worms in developing countries (Jimenez *et al.*, 2004). According to Alouini and Jemli (2001), a fertilized female *Ascaris* worm in the human intestine can produce 200,000 eggs per day. Conventional wastewater treatments transfer parasite eggs from wastewater to sludge by sedimentation and the ability for destruction is very low, consequently there is a high occurrence of these parasites in sludge. In Mexico City, the helminth ova commonly found in sludge are *Ascaris* spp., *Trichuris* spp., *Hymenolepis* and *Toxocara* (Table 1.8). Probability of infection varies according to sanitation coverage in developing countries and the use of wastewater and sludge for agriculture (Jimenez *et al.*, 2004). Table 1.13 shows transmission pathways and symptoms of some parasites found in sludge.

Table 1.13. Transmission pathways and symptoms by some parasites found in sludge (Jimenez *et al.*, 2004)

Parasite	Transmission paths	Symptoms
Ascaris spp.	Ingestion of contaminated water, food, or soil	Diarrhea, kidney, heart, bladder, pancreas, liver, and lung infections; intestinal obstruction; pneumonia
Trichuris spp.	Ingestion of contaminated food	Chronic hemorrhages
Hookworms (*Ancylostoma* spp. and *Necator* spp.*)*	Skin penetration or soil contact	Bronchopneumonia; anemia

Adult female eggs produce unembryonated eggs that develop a first, second, and/or a third larvae stage (Peng *et al.,* 2003). Infection in humans generally takes place via a fecal-oral route when humans ingest a second or third larvae stage. However, swallowing may occur in endemic *Ascaris* areas. *Ascaris* produce fertilized and unfertilized eggs which are distinct morphological forms. Fertilized eggs are elliptical in shape, varying from about 50 to 70µm by 40 to 50µm in size. Most fertilized eggs contain a zygote encased in a thick egg shell with four layers: an irregular outer coat produced by the female worm parent and three layers excreted by the embryo. Sometimes the outer coat is absent in fertilized eggs. On the other hand, unfertilized eggs are longer and narrower, from about 60 to 100µm by 40 to 60µm in size (Peng *et al.,* 2003). The characteristic of the egg shell of *Ascaris* eggs makes them difficult to penetrate and destroy; see Figure 1.2.

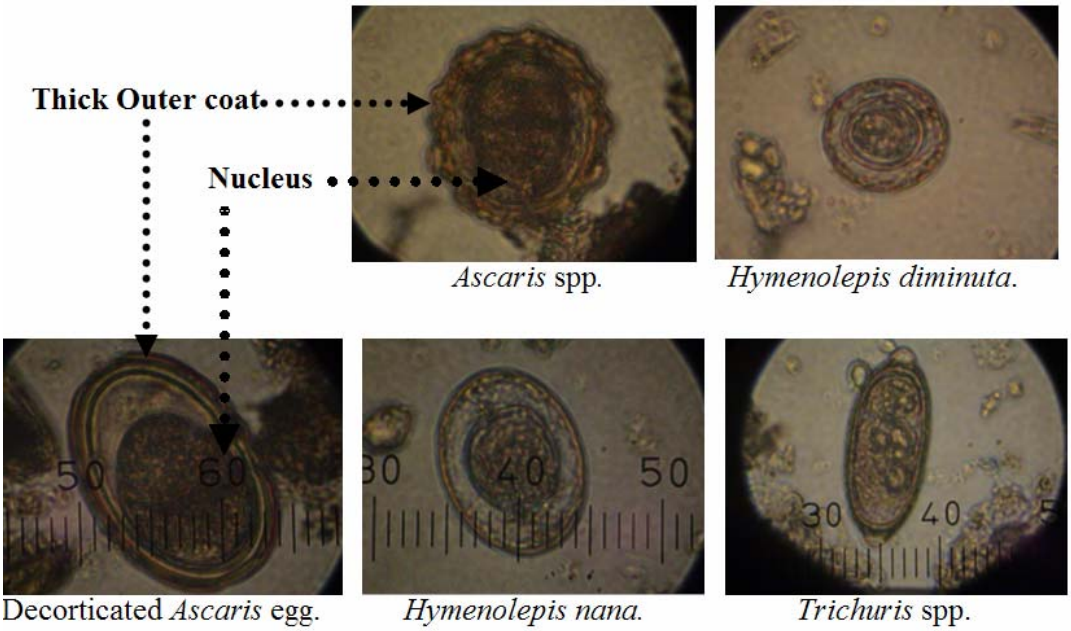

Figure 1.2. Various helminth ova in sludge from Mexico.

In Texcoco wastewater stabilization ponds, eggs seemed to survive up to 7 years in sludge (Nelson and Jimenez, 2000). Deactivating and destroying these parasites is a difficult task when the process does not include extreme changes in pH and temperature over 50^0C (Barrios *et al.,* 2004). Studies have shown the positive effect of organic substances in penetrating and dissolving lipids in the innermost layer of the egg. Also, volatile acids produced by some species during fermentation appear to be incorporated and utilized by the egg, as well as some chemicals that may affect key proteins, such as those found in the lipid layer of the egg (Barrios *et al.*, 2004). Determination of viability is very important when a very high occurrence of parasites is to be quantified. Inactivation of helminth eggs does not necessarily implicate destruction of the eggs; many eggs can still be found in sludge but their infectious stage is no longer possible because of damage to the nucleus of the egg. Viability is based on permeability of the inner coat of helminth eggs. Using some types of dyes, it is possible to determine permeability in helminth eggs. Thus, viable eggs are impermeable to some types of dyes, while non-viable eggs are permeable (De Victorica and Galvan, 2002).

Determination of helminth eggs are based mainly in two widely methodologies which are recommended by the US Environmental Protection Agency (EPA) and by the World Health Organization. In Nelson and Darby (2001) it is shown that viability can vary in the recovery process by these methodologies which rely on use of reagents in the different steps of the egg recovery. In this study it was concluded that among the reagents used, acid-alcohol had a greater impact in the egg viability in the extraction step; however, use

of this reagent has also shown to be an important factor in the process. Some given suggestions regarding this were that the extraction step could be substituted by a sieving step. A sieving step consisted on passing the sample through a 38µm mesh sieve instead of doing a hydrophilic extraction (acid-alcohol) and a lipophilic extraction (diethyl ether). A drawback remains in the low efficiency in cleaning samples which contain high concentrations of similar size sediments to the eggs (e.g. algae) that ultimately will interfere when counting at the microscope.

CHAPTER 3 - METHODS AND MATERIALS

In this project, the inactivation rates of helminth ova, fecal coliforms, and *Salmonella* spp. via solar drying and tilling treatments of biosolids were studied systematically. Sludge from Texcoco's wastewater treatment facility was used for the experiment because it originates from treated wastewater from Mexico City and has shown high pathogen and parasite concentrations. Because the effluent biosolids are on the order of 1% Total Solids, the first part of the experiment involved a "dewatering process." Three temporary dewatering beds were constructed of wood and a sand filter. Biosolids were dewatered over a period of 24 hours. The solar drying portion of this experiment was carried out at a greenhouse located at The Mexican Institute of Water Technology (IMTA) in Cuernavaca, Morelos, Mexico, and at the microbiology laboratory at this same research institution. Figure 1.3 is a view of the greenhouse and micro lab at IMTA.

Figure 1.3. The greenhouse (sludge treatments) and micro laboratory (assays) at IMTA.

3.1 Dewatering

It was found that wastewater treatment plants near Mexico City had no further practices in place to dewater or treat the liquid sludge produced from primary and secondary treatments. The main cause of this issue is because of the high incurring costs of a dewatering process. It has been reported that dewatering sludge depends on the process selected. About US$34 to 40 per dry ton is required for centrifuge, and about US$60 per dry ton for a belt filter press (Chen *et al.,* 2002). For this reason, the preliminary step in determining the die-off patterns of pathogens in biosolids in solar drying beds necessitated a dewatering process. Effluent sludge of approximately 1% TS from the treatment facility at Texcoco needed to be dewatered to achieve a product consisting of approximately 5% total solids. The minimum amount of dewatered biosolids required to study the pathogen reduction was estimated to be 450 kg. Although timeliness in dewatering the sludge was a critical issue, a second critical concern was trapping a measurable amount of helminth eggs to allow for tracking of the reduction pattern during the solar drying experiment.

A dewatering process consists mainly in separating the water present in sludge without evaporation (Chen *et al.,* 2002). By increasing the size of flocs (agglutination of small size particles) in sludge, solids can be retained. It is also known that size of bacteria and parasites eggs range widely in size. In Chavez *et al.* (2004), the range of sizes reported for fecal coliforms, *Salmonella* spp., and helminth ova are 0.7 to 1.5µm, 1.5 to 4µm, and

20 to 80µm, respectively. Thus, a dewatering process which ensured formation of flocs would also ensure agglutination of helminth eggs in the flocs. The dewatering problem was addressed by constructing three (3) wooden boxes, 2.0m wide x 2.0m long x 1.5m high. Three (3) 1" polyvinyl chloride (pvc) pipes with ½" holes drilled every 5cm were placed at the bottom of each box, which was then layered with 15cm of gravel and 20cm of sand. Pipes placed at an approximate 3% grade protruded outside of the boxes to allow for drainage of the effluent; see Figure 1.4. A wood frame with mosquito mesh was also placed above the sand to facilitate collection of the sludge and capture of the helminth ova eggs. An important characteristic of the dewatering box was that the process of infiltration was slow to minimize turbulence to avoid decreasing the size of flocs forming in the mesh. As the infiltration process was occurring, sludge would accumulate. To start the dewatering process, liquid sludge was pumped into each box in approximate volumes of $4m^3$ and filled at least three (3) different times to achieve the desired final quantity for the solar drying experiment. The dewatered biosolids, approximately 5%TS, were transferred using a clean shovel into barrels for storage and shipping to IMTA. The total desired quantity of sludge was dewatered within 24 hours; see Figure 1.5 and additional photos in Appendix A.

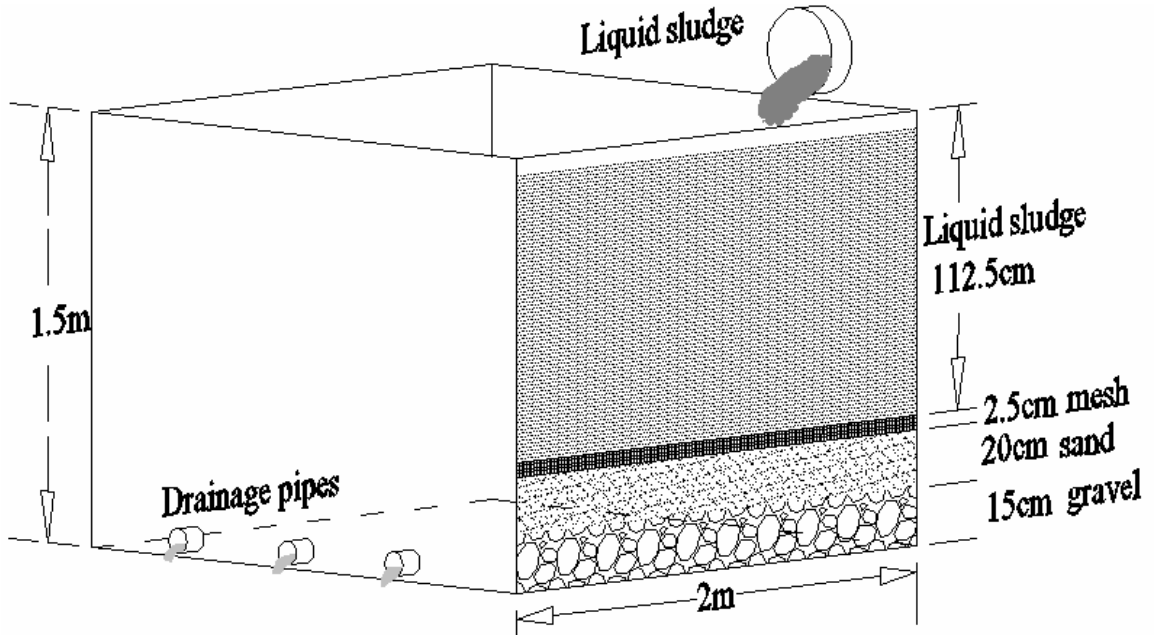

Figure 1.4. Wood box for liquid sludge dewatering.

Figure 1.5. Steps of sludge dewatering at the Wastewater Treatment Facility from El Lago de Texcoco.

3.2 Experimental Solar Drying Bed Setup in Greenhouse

It should be noted that the greenhouse structure itself was not entirely impervious to moisture penetration. The wall nearest to the drying beds was made of mesh and allowed for natural ventilation and perhaps moisture penetration when it rained. Three sand drying beds, approximately 1.0 m × 0.8 m × 0.15 m deep (for biosolids drying), were constructed inside an existing concrete bed of a greenhouse structure at IMTA, see Figure 1.6.

The sand drying beds were lined at the bottom with 15 cm of gravel, and had an upper layer consisting of 20 cm of sand. The concrete beds had pre-existing drainage holes which led to the floor of the greenhouse. Two type K thermocouples were placed in each bed at depths of 1 cm and 5 cm, and three (3) T107 probes (ceramic thermocouple, Campbell Scientific, Logan, Utah) were placed at 10 cm above the top layer of sand. One water content reflectometry probe, CS616 (Campbell Scientific, Logan, Utah), was placed diagonally in the biosolids layer thickness to account for an average measure of the thickness. After about 10 days, because of the biosolids drying and tilling treatments that caused a decrease in mass volume and because the sensor was not exposed to the air, the probe was placed horizontally at the surface of the sand layer and staked in place at the head of the probe; see Figure 1.7. This probe was utilized for the qualitative tracking of the decrease in biosolids moisture content. The biosolids dewatered at Texcoco WWTP were stirred in their barrels to attempt to make a homogenous mixture, then placed onto

each experimental drying bed at a depth of 15 cm (to ensure at least 10cm of dried biosolids). Beds were labeled as follows: Bed 1 received moderate tilling, i.e. three (3) times a week; Bed 2 received intensive tilling, i.e. tilling five (5) times a week; and Bed 3, which was used as a control, had no tilling applied. See photos in Appendix B.

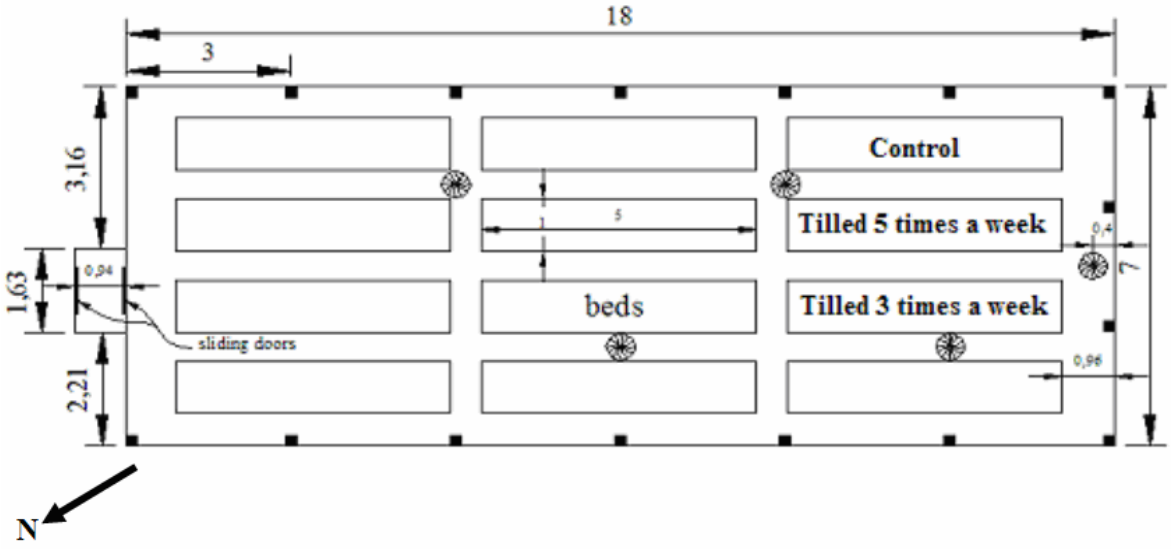

Figure 1.6. Top view of existing concrete beds in greenhouse at IMTA. Dimensions given in meters.

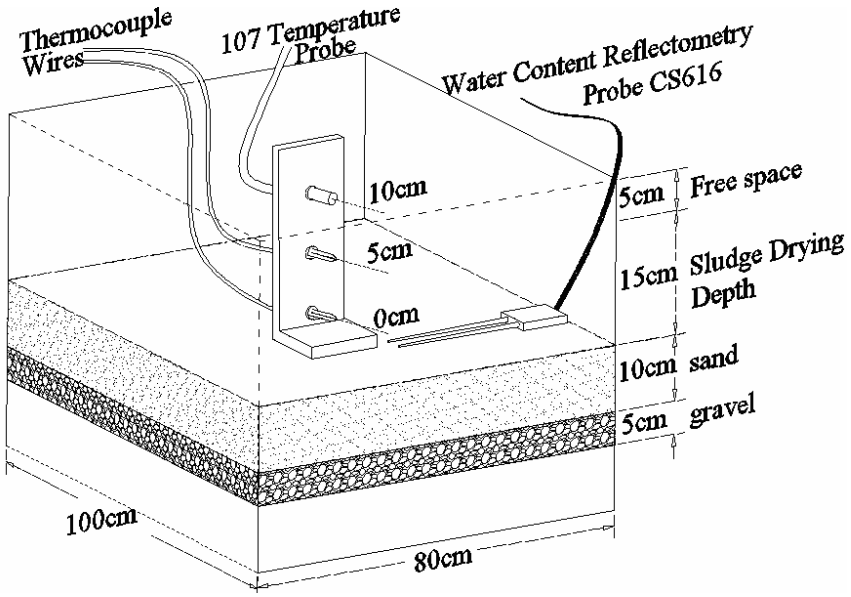

Figure 1.7. Dimensions and sensor setup in beds for sludge treatment.

3.3 Weather Station and Sensors

Air temperature and relative humidity inside the greenhouse were measured using a HMP45C combo sensor (Vaisala, Woburn, MA). Solar radiation (inside the greenhouse) was measured with a net-lite pyrometer (Campbell Scientific, Logan, Utah). The moisture content and temperature of the biosolids for each bed was also measured continuously at intervals of 10 seconds; the data was averaged and stored hourly in a Campbell Scientific CR10X datalogger (Campbell Scientific, Logan, Utah). This environmental data was directly downloaded to a computer for analysis. Environmental data pertaining to conditions outside of the greenhouse were measured and recorded by an automated weather station maintained by the National Meteorological Service (Servicio Meteorológico Nacional) of Mexico. Thus, data for the duration of this field experiment was acquired from the National Meteorological Service of Mexico and used for comparison with measurements inside the greenhouse. The percent total solids in each drying bed were determined when the microbial samples were performed for assay by weighing and drying the samples in an oven at 104^{o}C for 24 hours.

3.4 Sampling and Tilling Treatments

Tilling treatments were applied to beds 1 and 2. Bed 1 was tilled three times a week (moderate tilling), bed 2 was tilled five times a week (intensive tilling), and bed 3 was used as a control, with no tilling treatments. Tilling was done manually with a hand tool. Composite samples were collected from each drying bed on sample days according to the

EPA's sludge sampling guidelines (US EPA, 1989). Grab samples were taken from different points within each bed as well as at different depths in an attempt to get a representative sample for each bed in order to create one (1) composite sample for assaying. Samples were collected in sterile plastic bags and assayed within one hour; otherwise, they were kept at a temperature of 4^{o}C. Sampling and assays for fecal coliforms and *Salmonella* were scheduled on Days 0, 2, 9, 16, 23, 34, 41, 49, 65, and 70. For Day zero sampling, sludge was collected from one of the dewatering boxes at Texcoco WWTP, placed into a sterile plastic bag prior to storage in barrels and then put on ice and shipped to IMTA to be processed within 24 hours. In the case of helminth eggs, samples were taken on Days 2, 45, and 70 since it is known that destruction of eggs is more complicated and additional treatments are necessary to get the desired results. Also, a Day zero sample was considered for this parasite when the sludge was not dewatered to verify that the dewatering process did not modify the initial experimental numbers.

3.5 Microbiology Assays

3.5.1 Fecal Coliforms and *Salmonella* spp.

The Most Probable Number test (MPN) 9221 B, Standard Total Coliforms Fermentation Technique, is the typical method of practice for assaying indicator organisms in sewer sludge (American Public Health Association, 1998). This method was utilized during these experiments. During the first half of the experiment, Lauryl Tryptose broth was substituted for Lactose broth, as this was the media on hand at the microbiology lab at

IMTA. It was later verified that results from these two different media did not differ once assayed with both samples in one of the sampling days of the experiment. Three tubes for each dilution were employed. The MPN method for fecal coliforms takes approximately three (3) days to complete. A summary of the determination of fecal coliforms is described in Appendix F, Part A.

A modified version of EPA Method 1682: *Salmonella* in Biosolids by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium, Draft Document 2003 and the MPN protocol with three tubes for each dilution was employed to determine the *Salmonella* spp. density levels of the biosolids. The MPN protocol for *Salmonella* takes approximately six (6) days to obtain confirmation results. *Salmonella* from Trypticasein Soy Agar (TSA) plates were streaked in Tryptic Soy Agar and Lysine Iron Agar for confirmation, as described in the protocol. They were also confirmed using the Oxoid Latex Test Kit for *Salmonella* (Remel, Lenexa, Kansas). Numbers of *Salmonella* for Day zero (0) are smaller than actual indigenous numbers because only three (3) dilutions were processed due to timing and space in the laboratory. Appendix F, Part B summarizes the protocol followed for the bacteria.

3.5.2 Helminth Eggs

To determine the number of helminth eggs in sludge, the official Mexican methodology, NOM-004-SEMARNAT-2002, was used. This methodology was created based on the official (US EPA, 1994) methodology used in the US. Assaying of helminth ova is a direct enumeration technique and requires a series of sedimentation and flotation processes in order to enumerate the parasite.

As a result of the high numbers in wastewater from Mexico City, some modifications to the protocol were needed in order to get higher egg recuperations from samples. For example, for a mass of biosolids containing total solids up to 75%, a hydration period of 24 hrs was suggested to help separate the strong agglutination of solids before initiating the assaying process. Experience in counting and identifying these parasites is a critical key for obtaining realistic numbers. Because assays of helminth ova are tedious and time-consuming, it was decided to simply perform assays during three different sampling days in the experiment that would allow for demonstration of an inactivation trend in the three beds. Appendix F, Part C describes the procedure of helminth ova recuperation.

CHAPTER 4 - RESULTS

4.1 Environmental Data

Environmental parameters measured inside the greenhouse are compared with that of the outside; see Table 1.14. Also, Figure 1.8 shows the daily average relative humidity, air temperature, and the daily cumulative hourly measurements of the solar radiation in the 70 day period of the experiment. Figure 1.9, Figure 1.10, and Figure 1.11 show the trends of daily average temperature of biosolids layer in the treatment beds. The general trend is that the temperature increases as moisture from the biosolids declines. In the tilling treatment beds, temperature rises after Day 10, while the control bed (no tilling) shows an increase after Day 20.

Table 1.14. Summary of environmental information relative to the greenhouse at IMTA, April – June 2005.

	Indoor Air Temp °C	Outdoor Air Temp °C	Indoor Daily Solar Radiation (kw/m^2)	Outdoor Daily Solar Radiation (kw/m^2)	Indoor RH (%)	Outdoor RH (%)
Max	41.95	35.4	6.2	7.5	91.8	100
Min	16.59	15.4	1.9	3.6	8.44	12.0
Average	28.12	25.1	4.9	6.2	35.96	45.9

Transmittance of greenhouse cover = 0.74

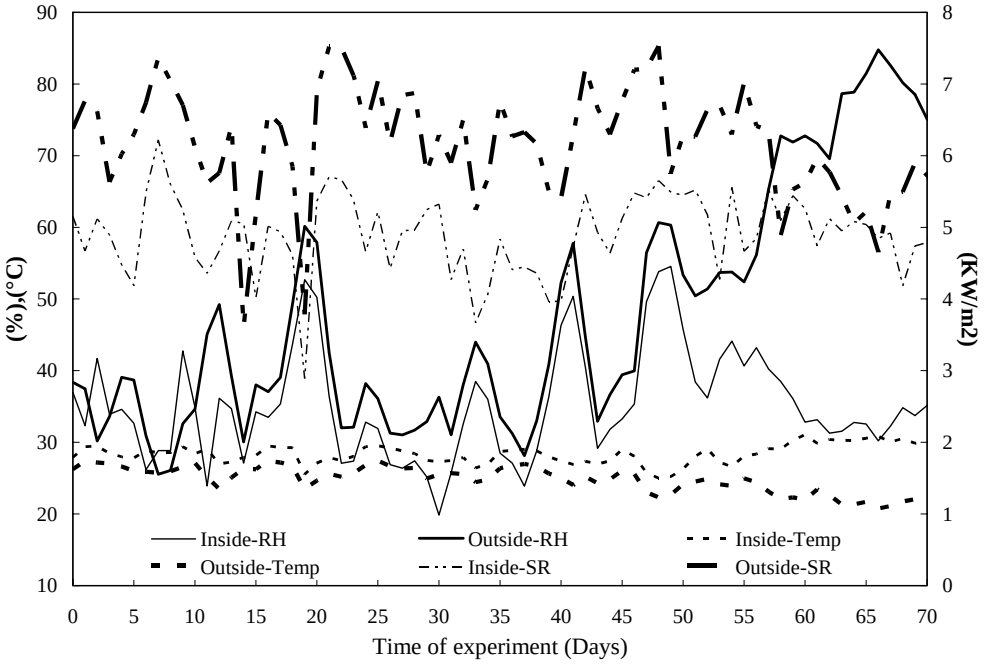

Figure 1.8. Daily average relative humidity, air temperature, and daily cumulative solar radiation.

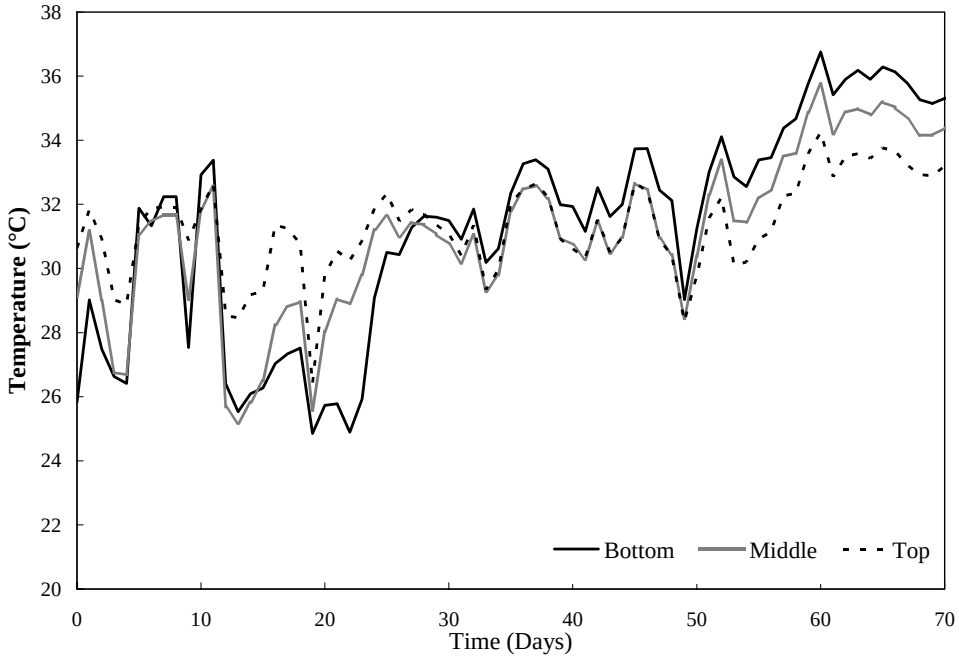

Figure 1.9. Daily average temperature in biosolids layer of the control bed.

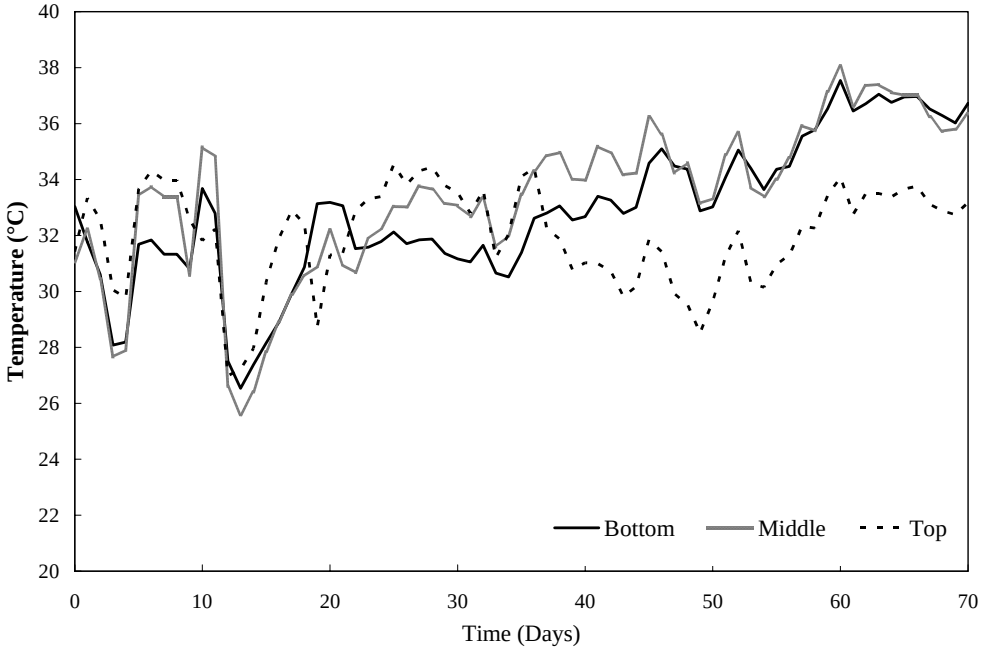

Figure 1.10. Daily average temperature in biosolids layer of the moderately-tilled bed.

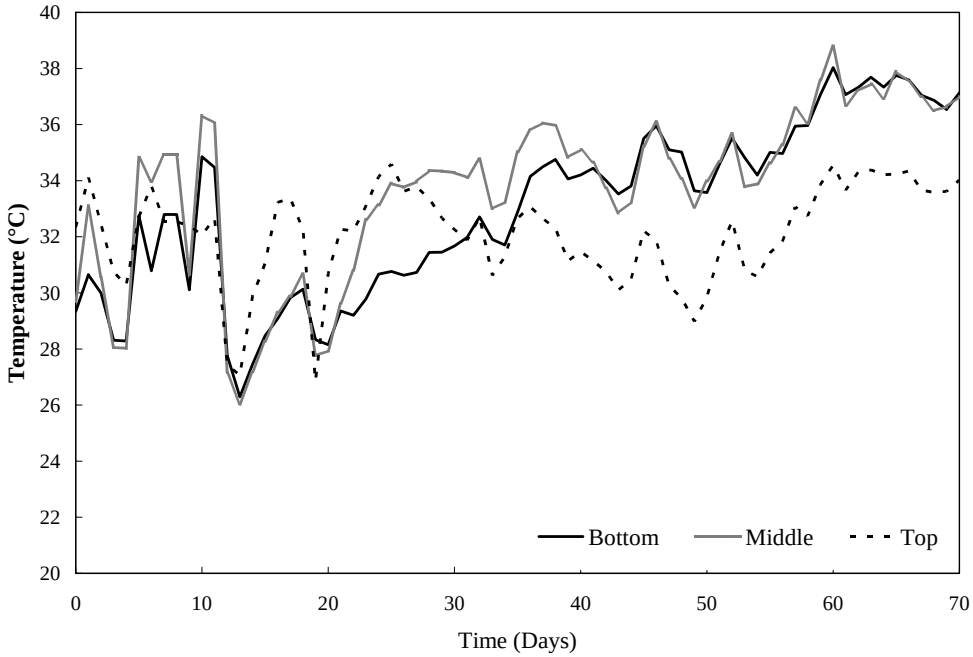

Figure 1.11. Daily average temperature in biosolids layer of the intensively-tilled bed.

Temperatures for the biosolids in the control bed for Days 1, 12, and 20 are represented in Figure 1.12, Figure 1.13, and Figure 1.14. In these graphs, we can see that temperature during the day ranged from 18°C to 46°C in the top layer. In the bottom layer, temperatures ranged between 21°C and 38°C. As moisture depletion was increasing, the biosolids layer was more uniform. However, at noon on Day 20, after rain events outside the greenhouse, temperature was lower in the bottom layer because of a cooling pattern that made air temperatures decline; otherwise, a similar trend as in Day 10 was likely to happen. Up to 40°C of temperature in an hour-long period was observed in days similar to Day 12 in the control bed.

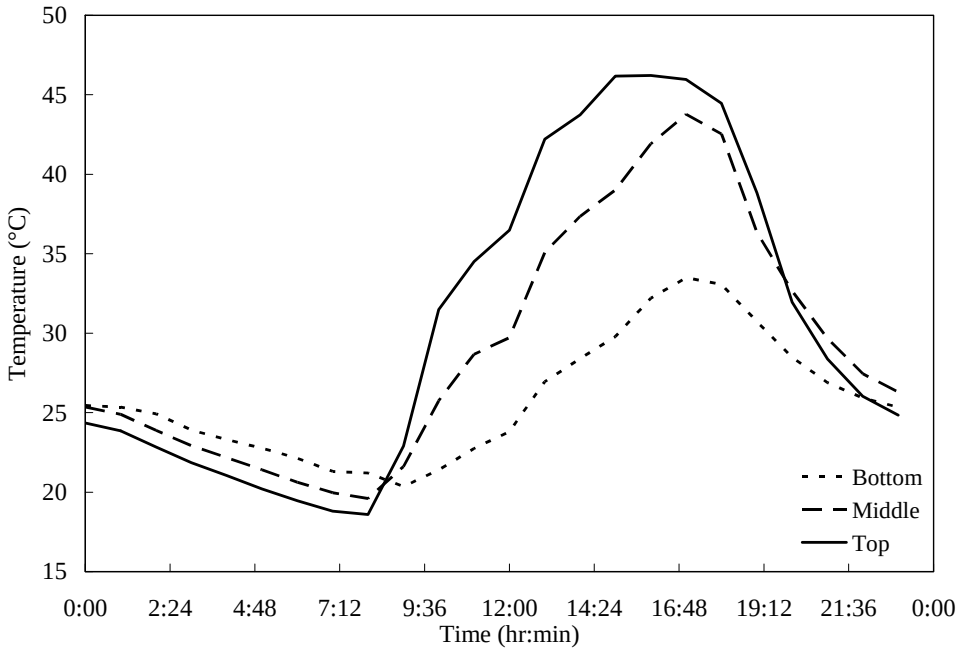

Figure 1.12. Temperature of biosolids layer in Day 1 of the control bed.

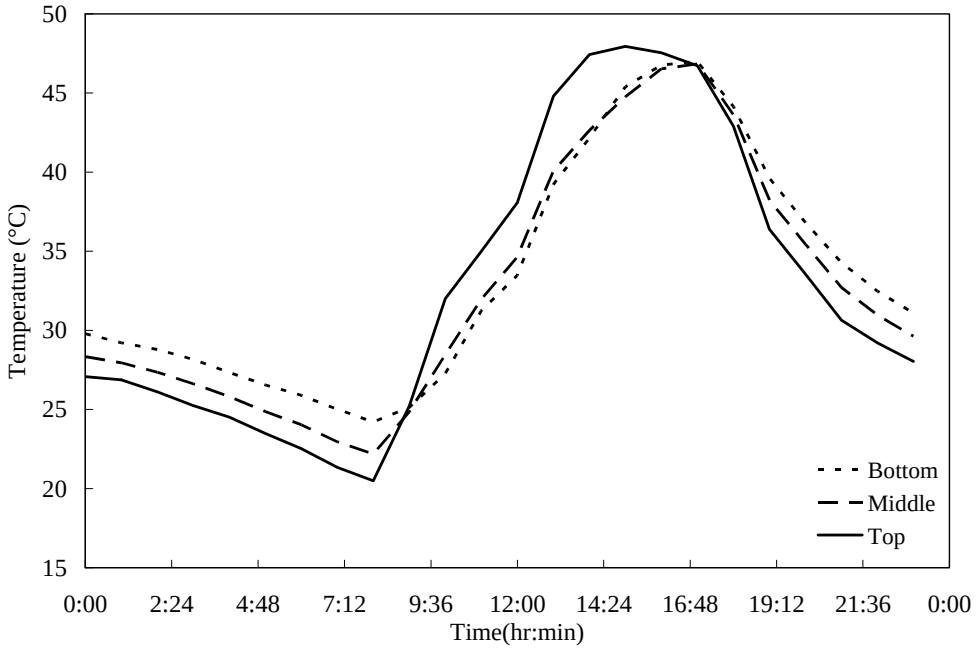

Figure 1.13. Temperature of biosolids layer in Day 12 of the control bed.

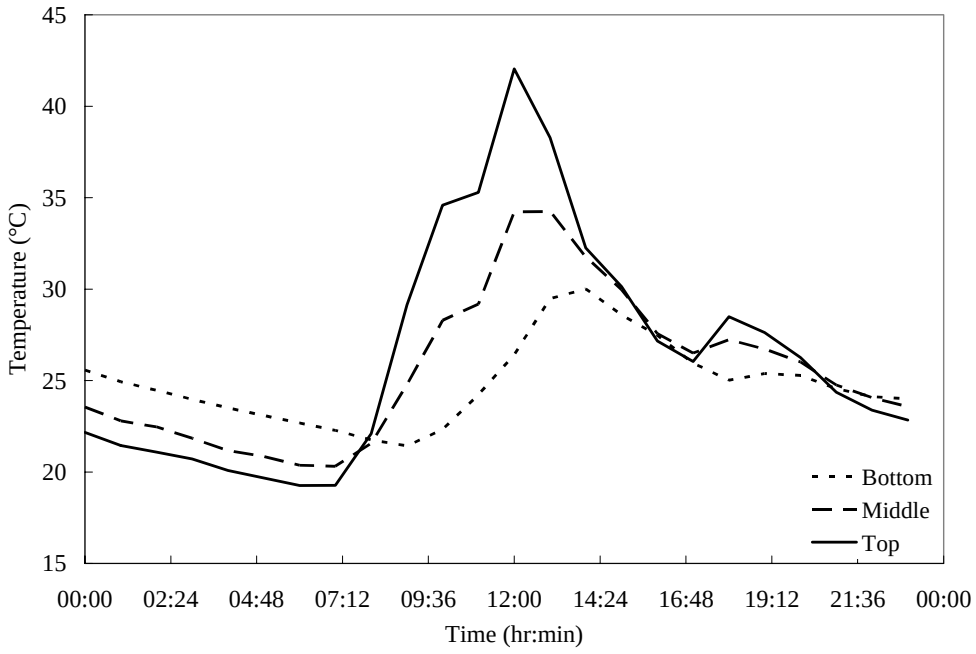

Figure 1.14. Temperature of biosolids layer in Day 20 of the control bed.

The temperature pattern of the biosolids layer in the intensively-tilled bed was similar to the control bed. The difference resides in that higher temperatures were experienced in the tilled beds. Up to 40°C in a period of two hours was observed after Day five. Figure 1.15 through 4.10 show the temperature trends in a certain day.

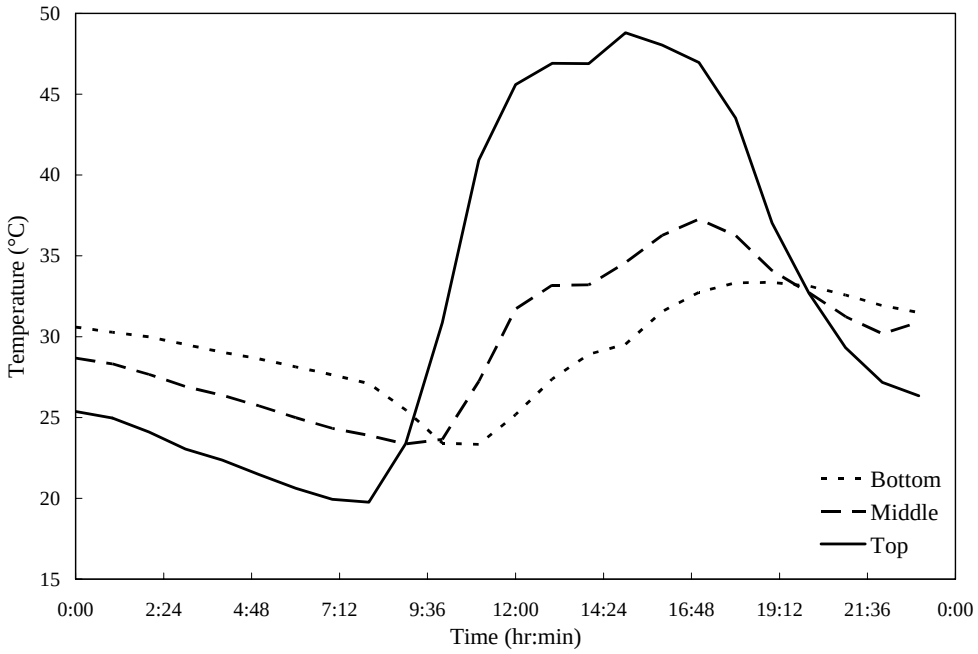

Figure 1.15. Temperature of biosolids layer in Day 1 of the intensively-tilled bed.

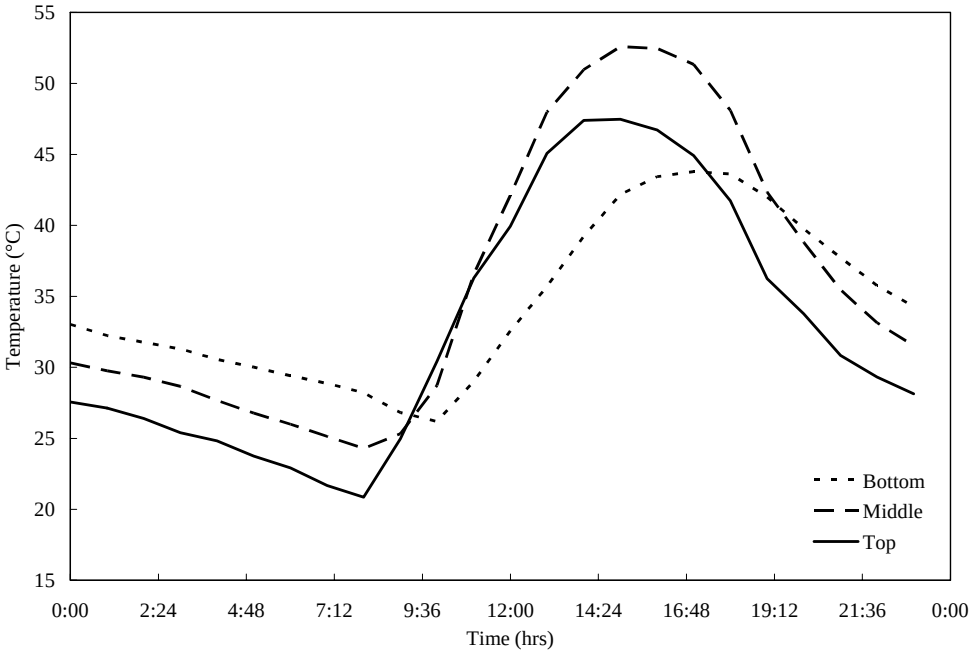

Figure 1.16. Temperature of biosolids layer in Day 12 of the intensively-tilled bed.

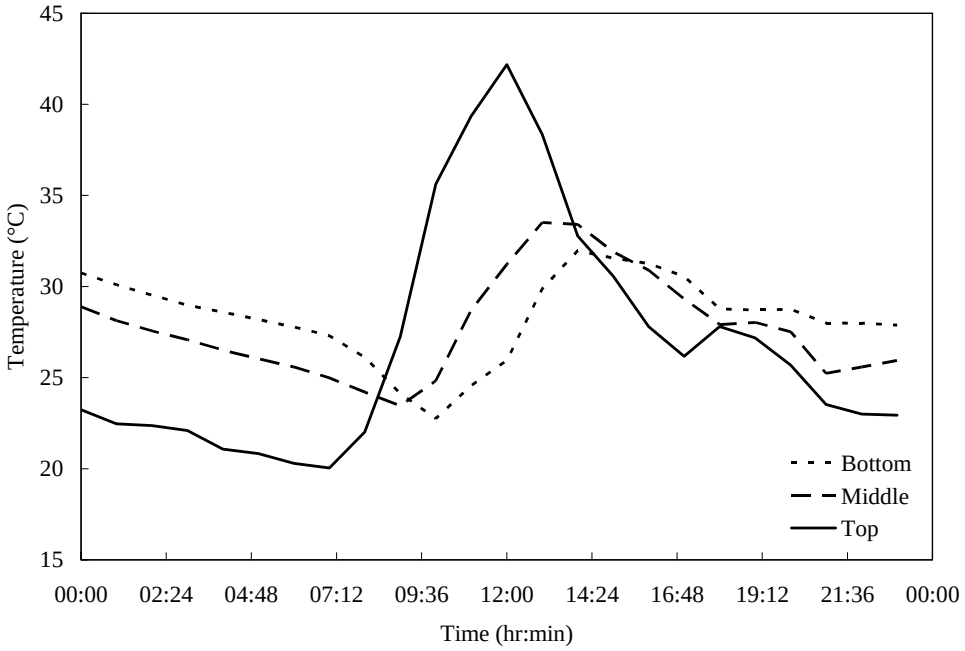

Figure 1.17. Temperature of biosolids layer in Day 20 of the intensively-tilled bed.

4.2 Drying Pattern of Biosolids

In this study, the biosolids which received moderate and intensive tilling treatments dried to nearly 90% and 100% total solids within 14 and 16 days, respectively.

Table 1.15 contains %TS data for the bed treatments. The biosolids which received no tilling took 27 days, or almost two (2) times longer, to reach nearly 100% total solids. Solar drying field experiments conducted in the semi-arid and arid regions of Arizona have shown that fecal coliform reduction follows the trend of moisture reduction within the biosolids (O'Shaughnessy et al., 2005).

The delay in reaching 100% total solids for the control bed may have been due to the high levels of relative humidity sustained inside the greenhouse; see Figure 1.18. The combinations of high humidity, reduced solar radiation (inside compared to outside values), limited air circulation, and rainfall events may have slowed the rate of evaporation. At Day 19 of the experiment, water content in the intensively-tilled bed was below 0.1v/v (water volume/total volume of sample) according to measurements from the probe sensor (see Figure 1.19). A relationship between the gravimetric moisture content and the sensor reading do not match an equal trend; however, a very close trend is seen in the moisture content depletion. This variation is due to the merely qualitative measure of the water content reflectometry probe.

Table 1.15. %Total solids for each drying bed as determined by gravimetric measurements.

Day	Control (Bed 3-No tilling)	Moderately- tilled (Bed 1-tilled 3x/week)	Intensively- tilled (Bed 2-tilled 5x/week)	Sampling Date
0	4.167	4.167	4.167	04/04/2005
2	5.795	5.602	7.551	04/06/2005
5	7.934	9.982	11.561	04/09/2005
7	8.192	10.686	10.638	04/11/2005
9	12.328	18.101	29.927	04/13/2005
12	25.163	54.032	48.748	04/16/2005
14	13.659	86.755	60.302	04/18/2005
16	27.759	70.939	96.629	04/20/2005
19	53.542	55.383	97.711	04/23/2005
23	83.790	82.342	95.755	04/27/2005
27	95.095	94.660	96.722	05/01/2005
34	95.601	97.151	97.798	05/08/2005
41	93.693	94.535	95.159	05/15/2005
43	95.566	96.111	96.883	05/17/2005
51	96.280	97.989	98.050	05/23/2005
58	95.085	96.014	96.299	05/30/2005
67	96.115	97.511	97.044	06/08/2005
70	95.086	96.236	96.764	06/13/2005

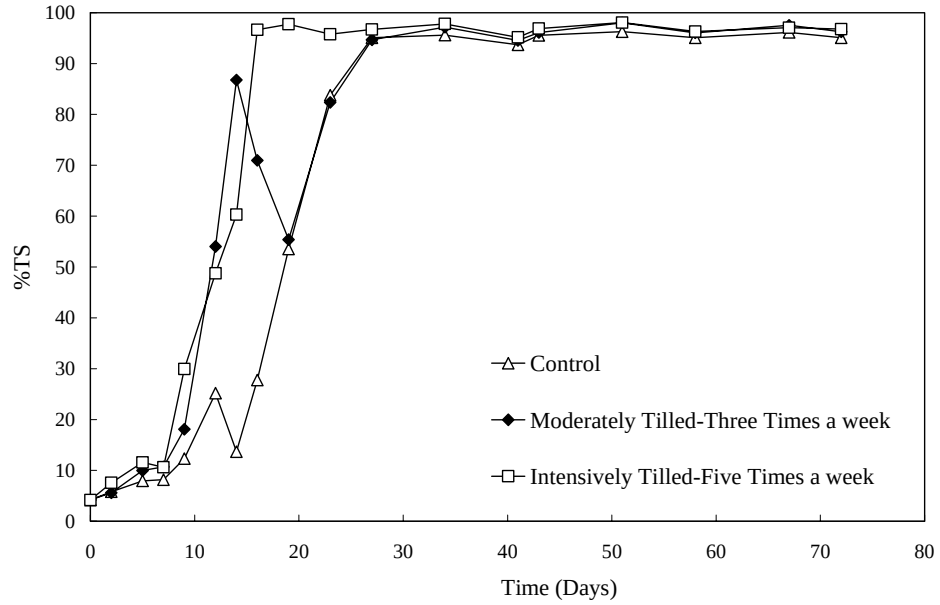

Figure 1.18. Rate of drying expressed as change in % total solids per day. %Total solids were measured by heating a known amount of biosolids sample in an oven at 104°C for 24 hours.

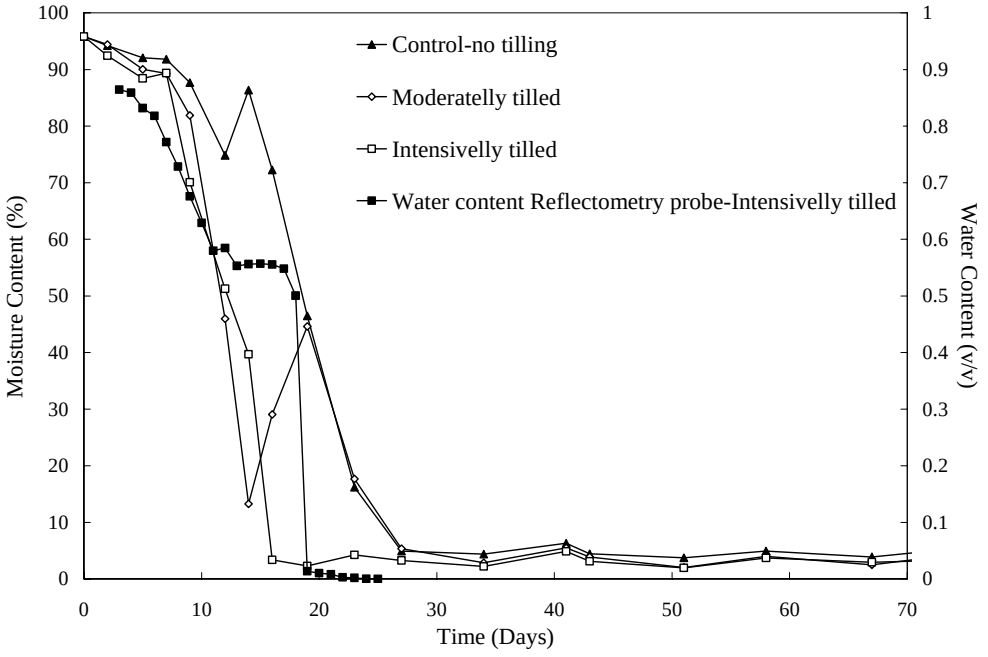

Figure 1.19. Moisture content in % total solids and volumetric water content (intensively tilled).

4.3 Microbial Results

4.3.1 Helminth Eggs

While helminth ova are comprised of three different varieties, and all three were observed, only *Ascaris* eggs were counted. This decision was based on the fact that the *Ascaris* eggs numbered the highest by far in the biosolids utilized and because *Ascaris* are highly persistent.

Table 1.16. Results of microbiological laboratory assays of *Ascaris* eggs sampled from experimental beds in the greenhouse at IMTA.

Day	Control (Bed 3-No tilling)	Moderately tilled (Bed 1-tilled 3x/week)	Intensively tilled (Bed 2-tilled 5x/week)	Sampling Date
2	58	94	138	4/6/2005
45	60	62	40	5/20/2005
70	22	4	0	6/13/2005

Numbers in eggs/4g dried weight of biosolids

Results for these parasite assays support the findings in the literature that high numbers of Ascaris eggs can be found in fresh sludge in Mexico; see Table 1.10. As observed in Table 1.16, day 0 numbers varied from bed to bed, more than likely due to the lack of homogeneity of the egg population within the biosolids as a result of the dewatering process at Texcoco WWTP (quick dewatering was antagonistic to filtering “in” helminth eggs) and in part due to error inherent in the involved assay process for their enumeration. In bed 2, the intensively tilled treatment, the number of *Ascaris* eggs was 138 eggs/4g dried weight of biosolids. In bed 1, which received moderately tilled

treatments, 94 eggs/4g TS were initially counted. Finally, in bed 3, the control bed, 58 eggs/4g TS were detected at the start of the experiment. Because of this discrepancy, a "fresh" sample of biosolids was retrieved from the Texcoco WWTP and carefully assayed; the enumerated number was 109 eggs/4g TS.

Tilling treatments were found to be very effective in reducing the number of detectable *Ascaris* eggs. The reduction rate for the intensively tilled, moderately tilled, and control beds were 0.88, 0.55, and 0.22 eggs/4g TS day-1, respectively. While the rates are not significantly different (p=0.226), the trend is apparent. The graph in Figure 1.20 shows the rate differences among tilling treatments for helminth ova by plotting the normalized value of the *Ascaris* eggs (N_t/N_o) over time, where N_t = the number of *Ascaris* eggs present at time (t) and N_o is the original number of helminth ova detected at Day 0.

Ascaris eggs in the intensively-tilled bed were reduced at a rate that was four (4) times greater than the control bed. Performing moderate tilling reduced the rate of *Ascaris* egg destruction by approximately 2.5 times that of the control bed. It is also evident that tilling reduced the final number, N (70) for each bed, to very minimal values; i.e., 0 and 4 eggs/4g TS for the intensively-tilled and moderately-tilled beds, respectively. Comparatively, the control bed contained 22 eggs/4g dry weight biosolids on Day 70.

Quality assurance and quality control is limited with this type of assay due to the limitations involved in separation by flotation and sedimentation of eggs from organic

material in the biosolids. Bowman *et al.* (2002) report that *Ascaris* egg recovery assays do not generally have an accuracy rate over 50%.

Because the assay for helminth ova is time consuming, it can be quite costly; therefore, it would be valuable to find a meaningful indicator microorganism that is more accurate and less costly and time-consuming to assay. If fecal coliforms can serve as an indicator of helminth ova, they would be a more economical method of determining pathogen disinfection levels.

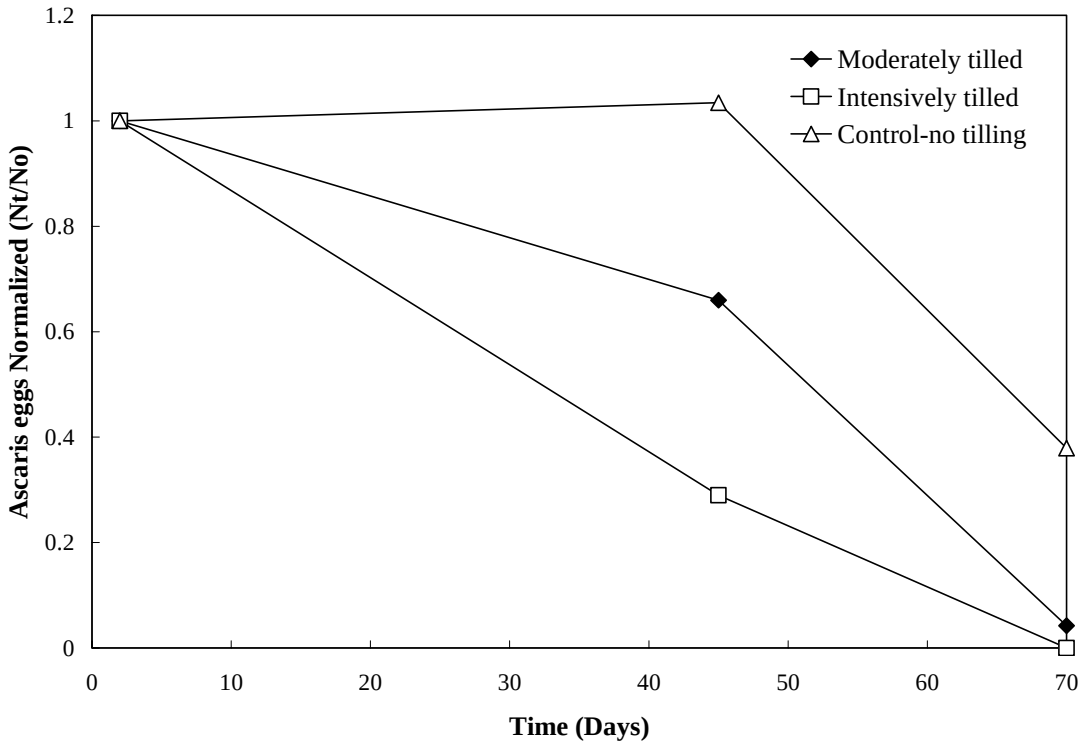

Figure 1.20. Normalized *Ascaris* eggs counts over time for the experimental beds at IMTA.

4.3.2 Fecal coliforms

The initial level of fecal coliforms, N_o, 5×10^6, was typical for biosolids produced at a secondary wastewater treatment plant. After two (2) days in the drying bed, the fecal coliforms levels declined by 1, 3, and 2 logs in the control, moderately-tilled, and intensively-tilled beds, respectively. The probable reason for the sharp decline is a response to the change in environment. After Day 2, the fecal coliform populations in all beds increased, nearly approaching No levels. In all likelihood, the rise in population between Day 2 and Day 9 was due to the acclimation of the bacteria to their new environment. Stable population numbers were sustained throughout Day 16 and then began to decline seven (7) days later; the reduction was 1 to 2 logs in each bed. On Day 34, the fecal indicators in each bed rose again. The outside weather station collected rain on Days 11, 17, 18, 19, 20, 38, 39, 40, and Days 45, 46, and 47, as shown in Figure 1.21. The elevated relative humidity corresponding to rainfall events may have been responsible for the sustained high population levels shown in the assays performed on Days 16 and 34. See below Figure 1.22.

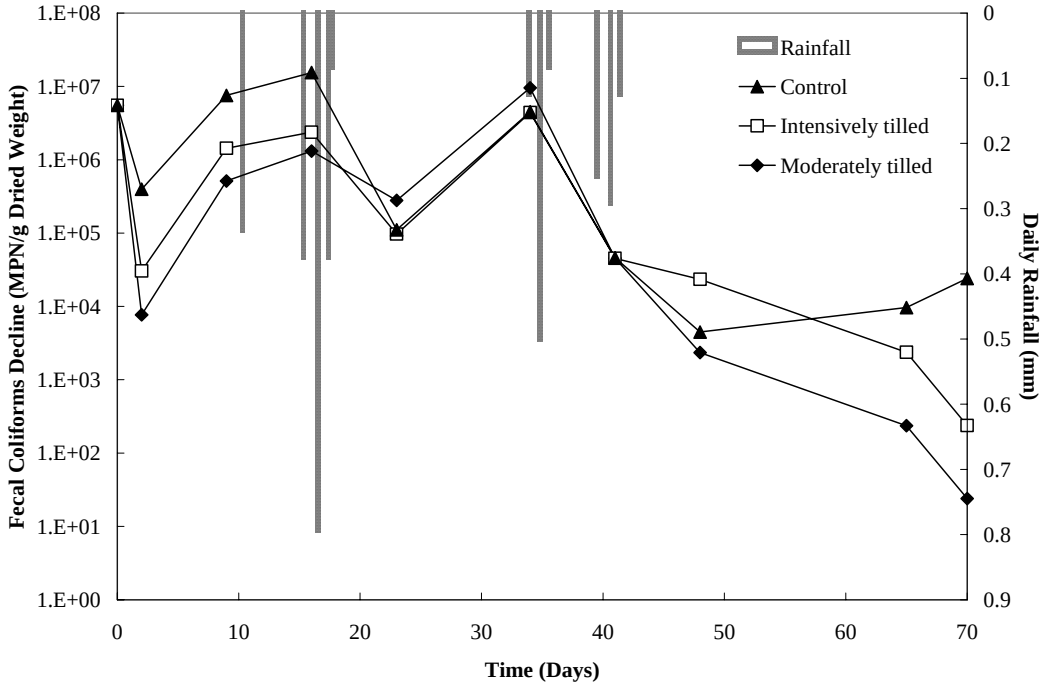

Figure 1.21. Fecal coliform density levels and rain events during the experiment.

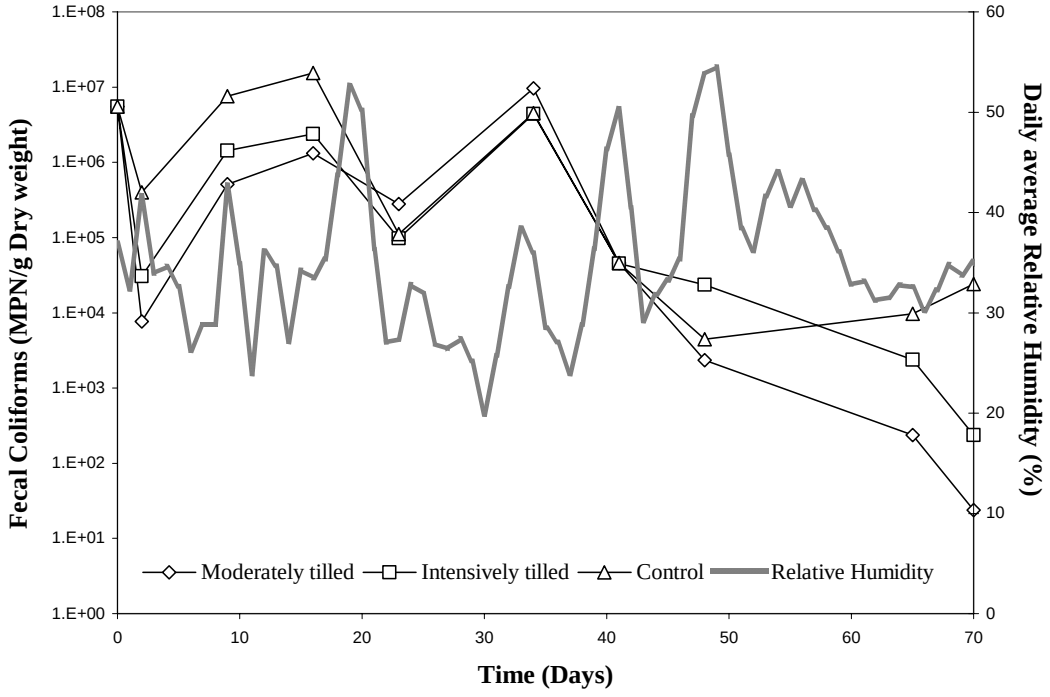

Figure 1.22. Fecal coliform density levels and relative humidity during the experiment.

After Day 34, the concentration of fecal coliforms decreased consistently in the tilled beds throughout Day 70 of the experiment. The inactivation rates were 0.10 MPN/g dry weight and 0.13 MPN/g dry weight for the intensively and moderately-tilled beds, respectively. The moderately-tilled bed reached Class A criteria (<1000 MPN/g TS) by Day 65, while the intensively-tilled bed reached Class A criteria by Day 70. The control bed did not reach Class A criteria during the experiment and began an upward growth trend after Day 48; see Figure 1.23. Density levels for fecal coliforms are also shown in Table 1.17 (underlined data meet Class A criteria).

Table 1.17. Fecal coliform density levels for the experimental drying beds at IMTA.

Day	Control (Bed 3-No tilling)	Moderately tilled (Bed 1-tilled 3x/week)	Intensively tilled (Bed 2-tilled 5x/week)	Sampling Date
	MPN/g Dry Weight			
0	5.52E+06	5.52E+06	5.52E+06	4/04/2005
2	3.97E+05	7.68E+03	3.05E+04	4/06/2005
9	7.54E+06	5.14E+05	1.44E+06	4/13/2005
16	1.55E+07	1.31E+06	2.38E+06	4/20/2005
23	1.11E+05	2.79E+05	9.71E+04	4/27/2005
34	4.50E+06	9.57E+06	4.40E+06	5/08/2005
41	4.59E+04	4.55E+04	4.52E+04	5/15/2005
48	4.47E+03	2.35E+03	2.35E+04	5/23/2005
65	9.68E+03	2.36E+02	2.37E+03	6/08/2005
70	2.42E+04	2.39E+01	2.38E+02	6/13/2005

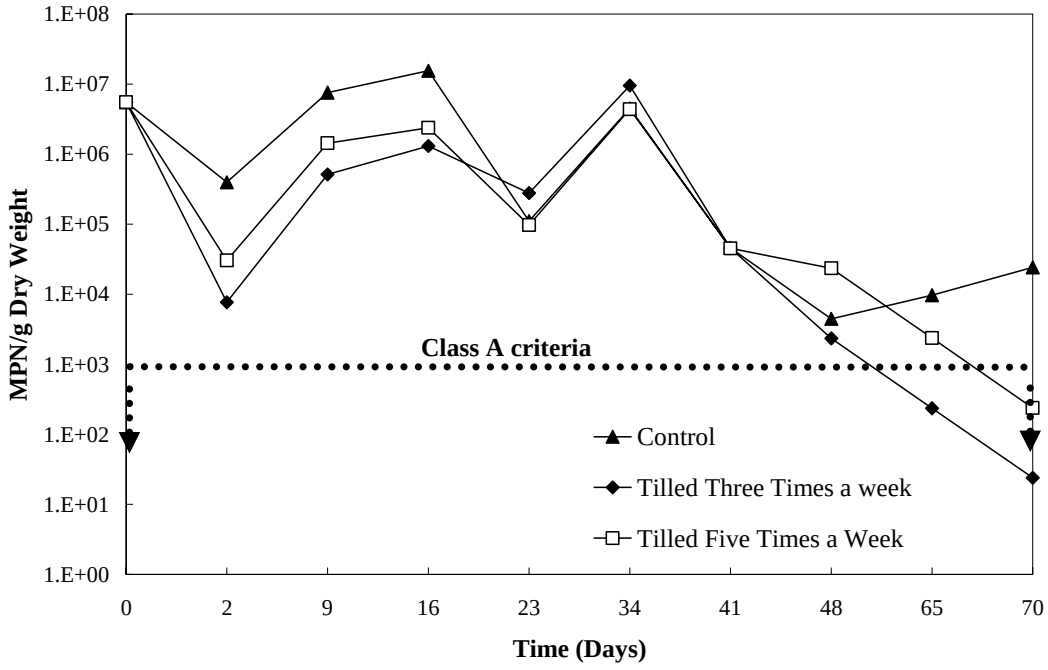

Figure 1.23. Fecal coliform density levels throughout the duration of the solar drying experiment, IMTA.

4.3.3 *Salmonella* spp.

The initial *Salmonella* levels in all beds were high. However, the rate of decline within the first 10 days of drying was dramatic; see Table 1.18 (underlined data do not meet Class A criteria). Both the moderately and intensively-tilled beds reached Class A criteria by Day 9 (<3 MPN/4g TS). By Day 16, all three beds had reached Class A criteria. However, the levels of Salmonella spp. periodically increased beyond this criterion for all three beds; see Figure 1.24 below. From Days 23 to 34, detection levels of *Salmonella* were 22 and 100 MPN/4g Dried Weight in the control bed; but in Day 41 it decreased again to 5 MPN/4g Dried Weight, until Class A levels were detected again on Day 49. For the tilled beds, small peaks were observed on Days 49 and 34 for the moderately and intensively-tilled treatments. The lesser increases in *Salmonella* spp. population can be attributed to spatial variability associated with sampling. The higher numbers, however, are more likely due to environmental influences, such as moisture penetration from rainfall or increased relative humidity. Similar results were demonstrated in Quartzsite and Green Valley, Arizona during the winter months when the relative humidity was high (>50%).

The significant temperature variations seen in the tilled beds were the product of the tilling treatments that added resultant energy to the biosolids. The porous dry biosolids reached high temperatures of approximately 55°C in the intensively-tilled bed. The control bed showed lower and more stable temperatures.

Table 1.18. *Salmonella* assay results for the solar drying experiment, IMTA.

Moderately tilled (Bed 1-3x)	Intensively tilled (Bed 2-5x)	Control (Bed 3-No tilling)	Day	Sampling Date
MPN/4g Dry Weight				
>2303.788	>2303.788	>2303.788	0	13/7/2005
328.5	582.7	1656.7	2	04/6/2005
ND	2.81	68.13	9	04/13/2005
0.203	0.257	0.519	16	04/20/2005
1.409	0.877	21.959	23	04/27/2005
2.182	3.886	100.417	34	08/5/2005
0.677	0.462	5.123	41	05/15/5
9.797	0.938	0.378	49	23/5/2005
0.373	1.195	0.379	65	06/8/2005
0.956	2.191	1.809	70	06/13/5

ND: Not detected.

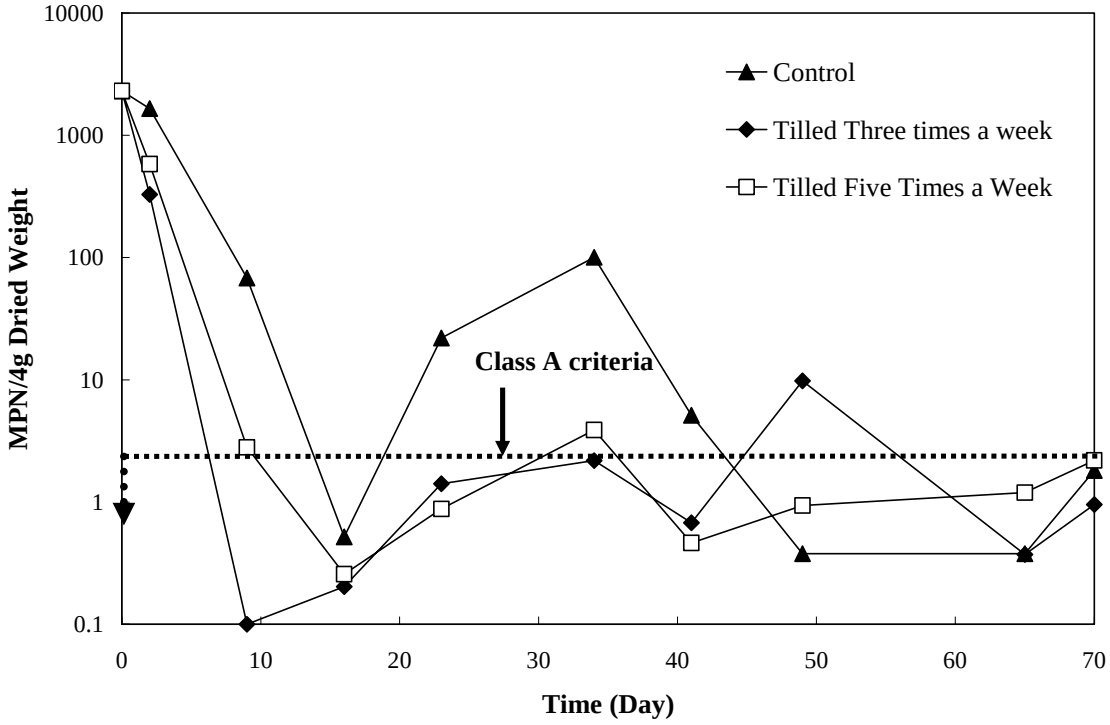

Figure 1.24. *Salmonella* die-off patterns for the solar drying experiment, IMTA.

CHAPTER 5 - DISCUSSION AND CONCLUSIONS

Solar drying of biosolids using sand beds in a greenhouse was effective in reducing pathogen levels in Mexico. Environmental parameters and tilling treatments were key factors in reducing the indigenous pathogens. Tilling treatments not only accelerated evaporation of the biosolids, but ensured rapid pathogen inactivation once high % total solids were reached. For example, reduction rates for helminth ova in the intensively-tilled bed was 0.88 eggs/4g TS day^{-1}, while in the control bed with no treatment, the reduction rate was one-fourth the rate, or 0.22 eggs/4g TS day^{-1}.

Salmonella spp. was found to be sensitive to moisture content and temperature variations. This fact was also supported previously in summer and winter experiments in La Paz County, Arizona (Choi *et al.*, 2005). On Day 9 of the experiment, *Salmonella* numbers were greatly reduced when biosolids reached 18, 30, and 12 %TS in the moderately, intensively-tilled, and control beds, respectively. Class A criteria biosolids were achieved in nine days for beds 1 (moderately tilled) and 2 (intensively tilled) with tilling treatments; however, this was not the case for the control bed. Regrowth was an issue that affected the control bed more. In the intensively-tilled bed, slight regrowth was observed just above the Class A criteria (3.8 > 3 MPN/4g Dried Weight). For the moderately-tilled bed, late regrowth was noted (9.7 > 3 MPN/4g Dried Weight) that could be attributed either to a tilling deficiency or just a hot spot in sampling. In the case of the control bed, a clear regrowth was detected (100 > 3 MPN/4g Dried Weight) mainly because of the

absence of tilling. Regrowth of these bacteria was also seen when the relative humidity increased to at least 75% after four consecutive rainy days. This affected the inside relative humidity, which rose proportionally to the incidence of rain. The interaction of both environments is attributed to the design of the greenhouse. The lower part of the greenhouse was protected by mesh, a material that allowed for free interaction of the air. Some correlation between Salmonella decline and %TS was observed; see Figure 1.25. On Day 20, the moderately-tilled and control beds were ~55%TS. On Day 23, %TS rose to 80% but *Salmonella* started increasing. Even thought S*almonella* did not display a sudden regrowth response with the decrease in %TS, the correlation is clear.

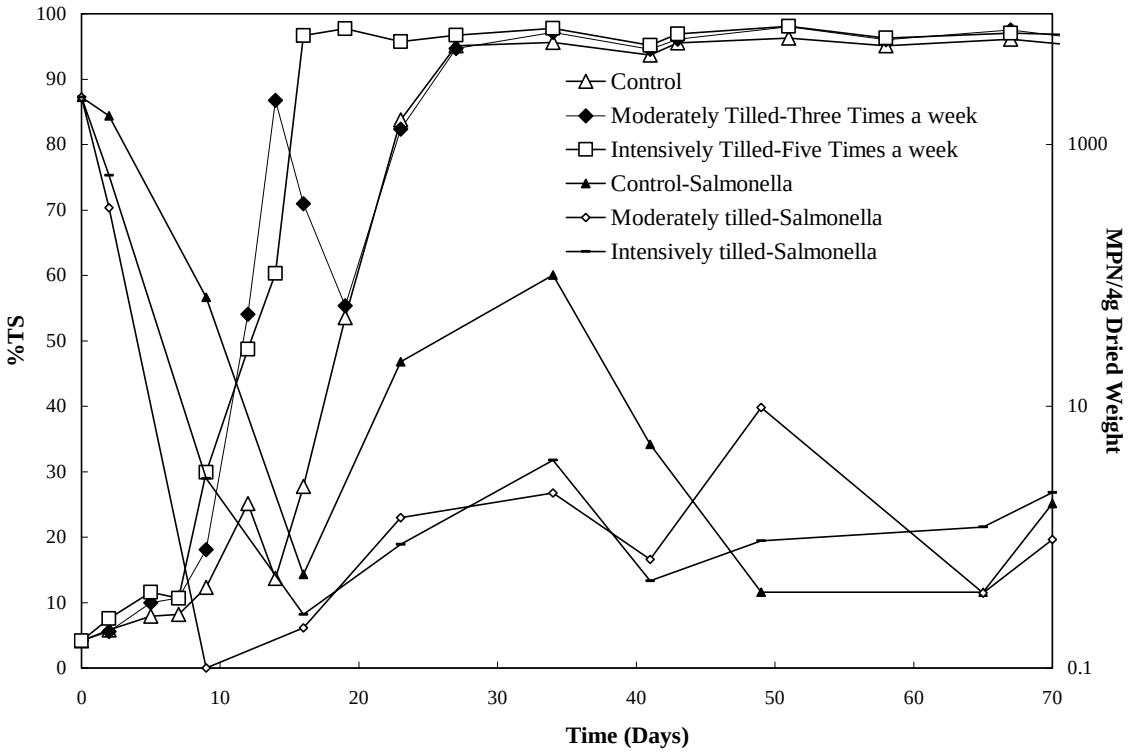

Figure 1.25. *Salmonella* spp. regrowth as a function of %TS.

In the case of fecal coliforms, numbers were maintained at high levels after evaporation of the sludge began. Temperatures approaching 40°C and high relative humidity in beds favored the presence of high fecal coliform numbers, until frequent tilling reduced the concentration enough to achieve Class A criteria at the end of the experiment. Solar drying beds in arid and semi-arid lands have shown that decay of fecal coliforms is strongly correlated with drying patterns. However, even biosolids at more than 90% total solids were still high in fecal concentrations (Choi *et al.*, 2005). The late response to a decrease in %TS could have made fecal coliforms levels rise. Despite reaching high %TS levels, up to 90%, fecal coliforms showed less dependency on moisture content because the increased concentration was sustained longer. However, at almost null moisture content, e.g., 90%TS, levels started to decline. A die-off pattern for fecal coliforms is displayed afterwards, as shown in Figure 1.26. Both the moderately and intensively-tilled beds achieved Class A criteria according to Table 1.1 on Days 65 and 70, respectively.

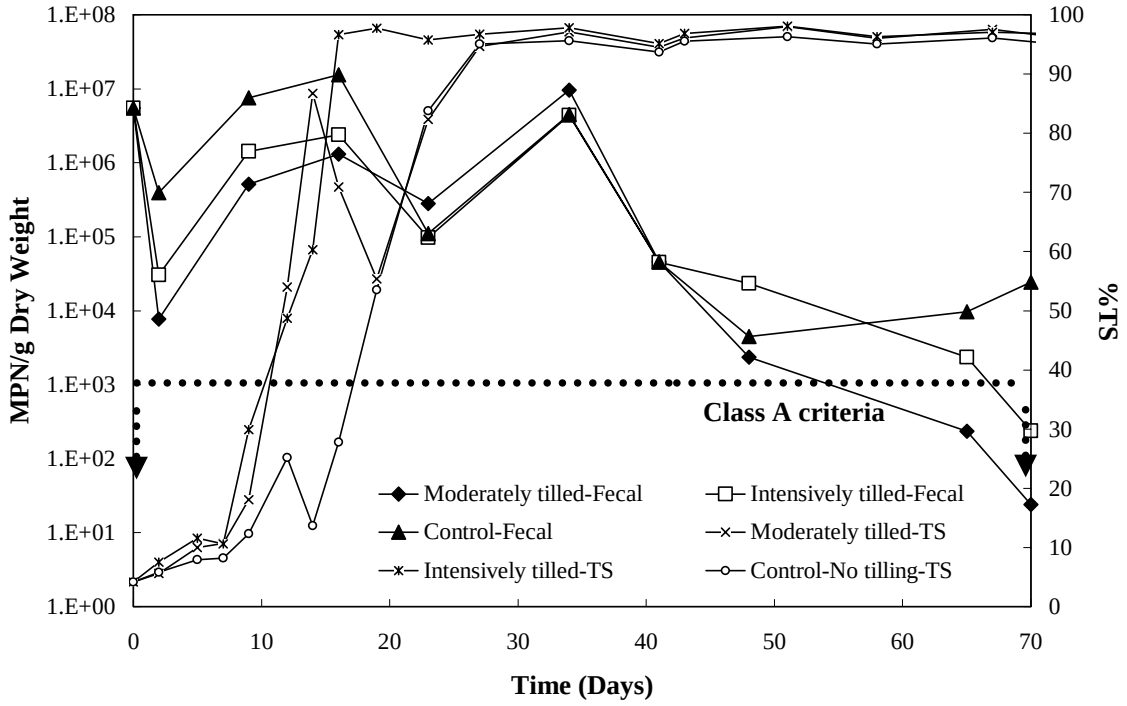

Figure 1.26. Late response for regrowth of fecal coliforms levels as a function of %TS.

It is also important to mention that both environments are completely different with regard to their various climatic parameters. Relative humidity in these semi-arid areas reaches a maximum of 21% in summer seasons, while in climates like Cuernavaca, Morelos, relative humidity approaches 100% at its highest. Thus, these two different extreme conditions of relative humidity may affect the acclimation of the pathogens by showing different die-off patterns. During the first 22 days of the experiment, maximum relative humidity reached at least 70%, and concentrations reached up to 5 logs. During these days, rain was reported on Days 11, 16, 17, 18, and 19. Because rain could not penetrate directly into the beds, moist air was the only indirect impact inside the greenhouse. In addition, daily average temperatures in the beds were 35°C and daily

maximum temperatures reached 40°C; it is known that fecal coliforms find favorable conditions at temperatures around ~ 40°C. However, tilling shows its effect over time if relative humidity does not increase sharply again. After Day 39, relative humidity went up to 80% in five (5) consecutive days, which caused regrowth only in the control bed. Tilling treatments over time appeared to cause stress in the intensively and moderately-tilled beds. At some point, levels of the stressed pathogens were lowered even when relative humidity was significantly high.

From the literature and past experiences, *Ascaris* eggs have been shown to be the most resistant to destruction. In this experiment, only tilling treatments were effective in completely destroying these parasites. High temperature variations, early moisture removal, and desiccation along with frequent tilling treatments were necessary to inactivate the eggs and achieve Class A biosolids levels. Only intensive tilling 5x (five times) a week was found to be effective in reaching Class A criteria. An appropriate and efficient tilling and turning protocol were indispensable to ensure that biosolids in beds experienced desiccation. Also, early high %TS of ~96%, maximum daily temperatures higher than 40^0C, and intensive tilling and turning (up and over) were applied to beds, one of which reached Class A levels. The moderately-tilled bed failed to reach Class A criteria. It should be pointed out that the hand-tilling tool was thought to be ineffective because of its simple design, but because the scale of biosolids volume was small, it was easy to handle for tilling and turning. Over time, biosolids became very hard to break. After 90%TS is achieved, the biosolids mass has strong particle agglutination and becomes difficult to break. In the intensively-tilled bed, breakdown of the biosolids was

ensured in tilling treatments that increased the probability for air penetration in the most inner areas of the agglutinated particles. Achievement of Class A standards may fail if eggs are agglutinated in these areas. The thickness of biosolids in experimental beds was important for tilling enhancement and penetration of solar radiation. In the experiment, a biosolids thickness of at least 10cm was used and treatment applications were effective for this small scale. Larger-scale experiments will need to consider better tilling equipment to ensure the results of this experiment. In the field experiments that took place in La Paz County, Arizona, a culti-mulch rotary tiller that operates attached to a tractor was used for treatment of biosolids (Choi *et al.,* 2005). More reliable tilling equipments may result in more successful Class A criteria levels if these issues are taken into account.

For the case of bacteria, regrowth was observed after acclimation and optimal conditions were gained; however, for *Ascaris* eggs, destruction is irreversible. Environmental conditions with high relative humidity limited and slowed the drying process. Regrowth was also highly correlated with relative humidity. Fast inactivation rates of *Salmonella* and fecal coliforms are found in arid and semi-arid areas; e.g., Quartzite and Green Valley, Arizona, in the US. Very low relative humidity and very high solar radiation favored achieving a more efficient desiccation process in these field experiments.

Because very low *Ascaris* numbers are found in sludge in Arizona, an inactivation and destruction process would be difficult to track. However, it is expected that *Ascaris* eggs

can be destroyed faster and with less intensive treatments in climates in Mexico similar to arid and semi-arid areas in Arizona.

By graphing the inactivation of helminth eggs and fecal coliforms versus time as shown in Figure 1.27, we could state that both microorganisms could work as indicators in this particular experiment for their similar inactivation trends. However, regrowth of fecal coliforms makes it difficult to correlate its die-off pattern to that of helminth ova. At this point, helminth eggs could more easily work as an indicator for fecal coliforms in sludge rather than fecal indicators for helminth ova.

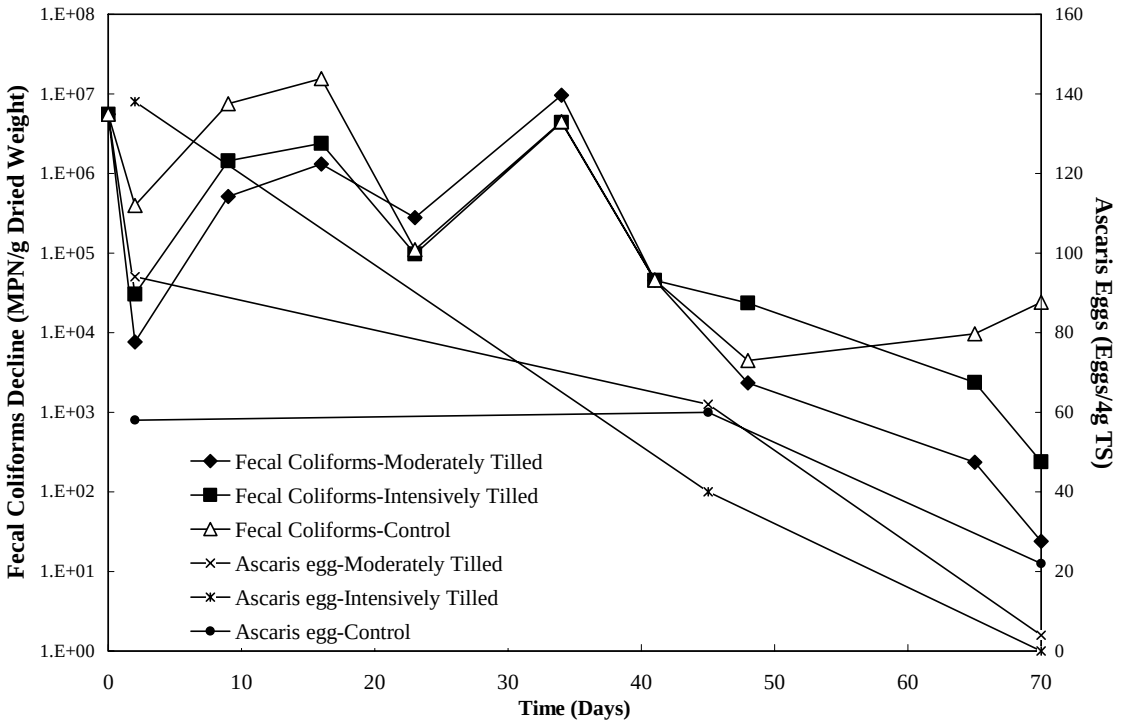

Figure 1.27. Fecal coliform and helminth ova density levels in the experiment.

Important parameters and points to consider are:

- High relative humidity interferes in achieving Class A criteria levels.
- High temperatures in drying beds are necessary for fast drying and significant variations over time for bacteria stressing.
- The higher the incoming solar radiation, the higher the temperature will rise. This is important in closed systems. Transmittance of cover materials is important in greenhouse structures for higher incoming solar radiation. The amount of incoming solar radiation depends also on the orientation of greenhouses.
- Closed systems will retain high temperatures and prevent direct incoming moisture from rain events.
- Insulation of greenhouses will improve the drying process in arid, semi-arid, and humid areas. However, capital and operation costs need to be evaluated.
- Tilling and turning treatments will improve and speed up the process of pathogen inactivation. Breakdown of biosolids to very small particles is necessary for desiccation. For *Ascaris* destruction, this is a critical point.
- Appropriate and more effective tilling tools will give better results in breaking down biosolids to a small size and improving their penetration to the innermost areas of particles.

Major findings in properly disposing of biosolids in most parts of Mexico will include:

Drying beds need to be adopted in wastewater treatment facilities for treatment of liquid sludge. A dewatering process in gravel-sand layer beds can be placed in treatment facilities to help incorporate biosolids disposal. Tilling and turning (up and over) should be definitely considered, since successful results in short periods are obtained by these treatments; additionally, they are cost effective. Large-scale biosolids disposal in Mexico should be inexpensive in order to successfully initiate this environmentally cost-effective activity. The solar drying and tilling technique is an adequate option when financial issues limit the possible use of a biosolids treatment technology. Because Mexico has more than 75% of its area in arid and semi-arid regions, utilization of solar drying beds in large-scale disposal areas is of major importance in addressing not just the different environmental contaminations (e.g., surface and groundwater contamination and soil contaminations), but also in addressing the improvement of the vastly-eroded agricultural lands. As of today, many of the wastewater treatment facilities operating in Mexico have not yet considered treatment of medium-to-large-scale solar drying beds for sludge treatment. Available land in the treatment plants will decrease the costs for drying and disposal of biosolids. One high incurring cost is the shipping of biosolids to the reuse location (e.g., agricultural lands, forests, landscapes, etc.). A high %TS, up to 80%, is recommended to reduce the volume of biosolids to be transported and decrease transportation costs. In Choi *et al.* (2005), it is stated that transportation costs will depend not only on the proximity of the wastewater treatment facilities (where sludge is being produced) to the sludge dehydration area, but also to the final reuse or disposal zone.

In the future, when constructing more treatment facilities, available land and appropriate areas for sludge treatment should be considered. One problem in the drying process is odor and vector attraction. If possible, isolating solar drying beds from urban areas will avoid potentially offending people nearby. Labor in the drying process is minor. The majority of wastewater treatment plants employ personnel for the various activities involved in the plant operation. These personnel can somehow incorporate and take over the activities of the solar drying beds (e.g., become operators for transportation and tilling schedules). Appropriate safety procedures for workers are important to prevent compromising their safety.

As shown in this work and in the winter experiments at La Paz County and Green Valley, Arizona, rain is a limiting parameter that will interfere in the treatment process when seeking the achievement of Class A levels. Easy-to-use, inexpensive greenhouse covers should be used to address the problem. It is crucial that the manager or the personnel of the treatment facilities are aware of the environmental parameters likely to happen in the next days in order to foresee and plan for the use of covers. In the arid areas of Arizona, treatment has been recommended to take place in summer seasons to avoid the problem; otherwise, the use of sensors for moisture detection should be set up to monitor moisture in biosolids. Trespassing on and consequent human contact with solar drying beds should be avoided to prevent human health endangerment and safety risks.

The use of chemical fertilizers in agriculture has become environmentally risky because of its frequent use for seeking high yields in non-fertile lands. Thus, biosolids can be substituted for synthesized fertilizers; their recycling can be greatly expanded to forestry, agriculture, and landscape use. Utilization of the biosolids will depend on the quality acquired and the compliance with the national regulations and guidelines in place for Mexico.

APPENDIX A - DEWATERING PROCESS

Figure A-1. Pouring liquid sludge in dewatering box.

Figure A-2. Dewatering process in wood box.

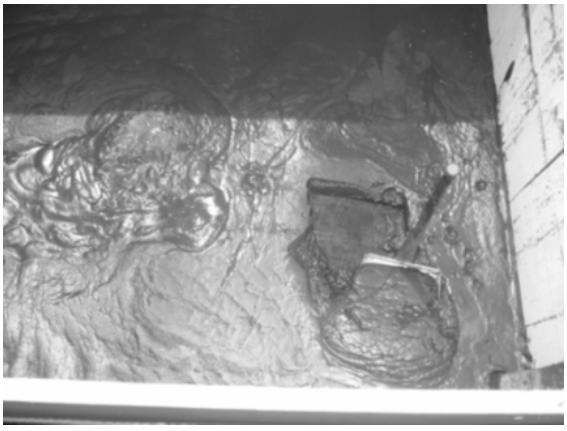

Figure A-3. Collecting sludge after dewatering.

Figure A-4. Mesh frame view after collecting sludge.

APPENDIX B - EXPERIMENTAL DRYING BEDS AT IMTA AND SENSOR SETUP

Figure B-1. Cleanup and preparation of beds.

Figure B-2. Pouring sludge to drying bed after shipping.

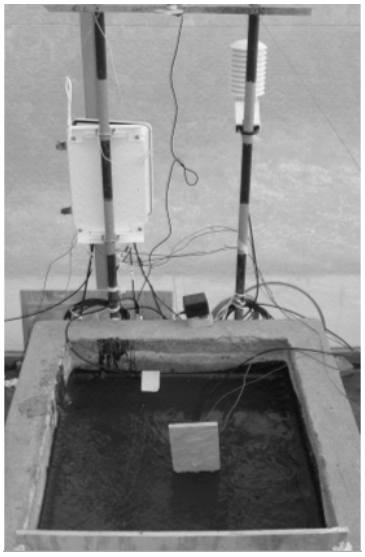

Figure B-3. Sensors in drying bed.

Figure B-4. Drying beds in greenhouse.

APPENDIX C - ENVIRONMENTAL SAMPLING AND TILLING: HAND-HELD RAKE AND BIOSOLIDS IN BEDS SHOWING PROGRESSIVE DRYING.

Figure C-1. Tilling with a hand-held rake.

Figure C-2. Showing tilling of biosolids.

Figure C-3. Sampling in bed 2 tilled 5x a week after tilling treatment.

Figure C-4. Sampling in bed 3-Control after tilling treatment.

Figure C-5. Bed 2 tilled 5x in Day 1 of the experiment.

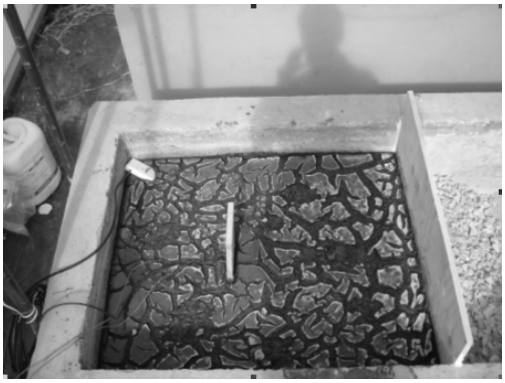

Figure C-6. Bed 1 tilled 3x in Day 1 of the experiment.

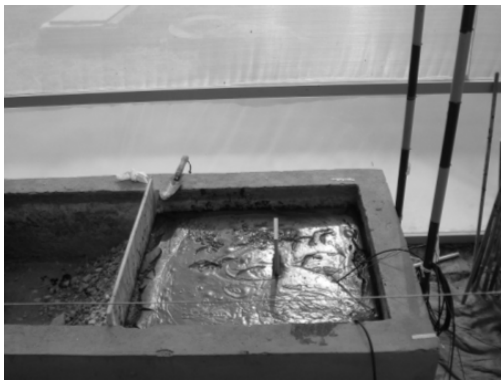

Figure C-7. Bed 3 Control in Day 1 of the experiment.

Figure C-8. Bed 3 Control in Day 9 of the experiment.

Figure C-9. Bed 1 Tilled 3x a week in Day 13 of the experiment.

Figure C-10. Bed 1 Tilled 5x a week in Day 13 of the experiment.

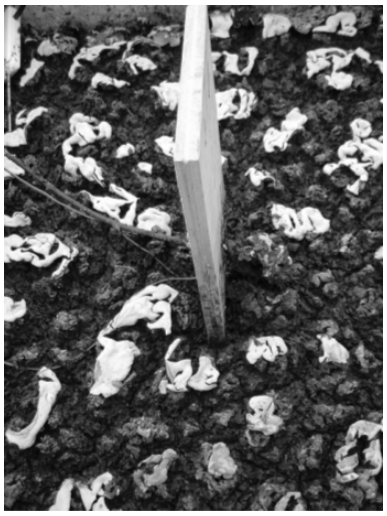

Figure C-11. Bed 3 Control in Day 13 of the experiment.

Figure C-12. Bed 3 Control in Day 56 of the experiment.

APPENDIX D - PICTURES SHOWING MICROBIAL ANALYSIS

Figure D-1. Transferring *Salmonella* spp. enriched in Rappaport Vassiliadis broth to Hektoen Agar plates to grow *Salmonella* colonies.

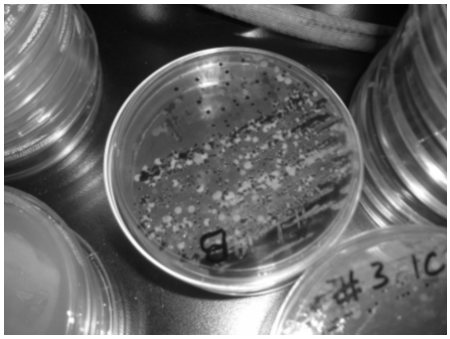

Figure D-2. Possible *Salmonella* colonies (black dots) grown in Hektoen media.

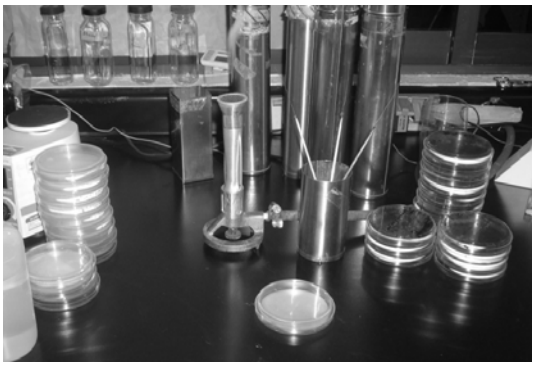

Figure D-3. Isolating pure black *Salmonella* colonies from Hektoen plates and transferring to Trypticasein Soy Agar (TSA) plates.

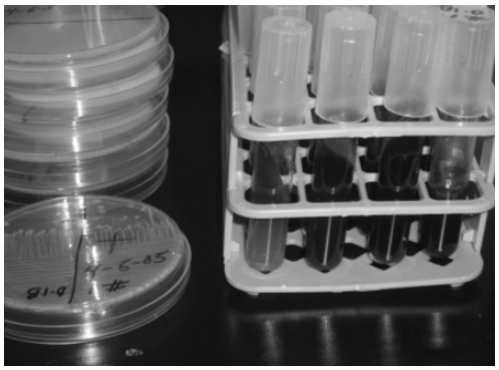

Figure D-4. Inoculating *Salmonella* colonies from Tryptic Soy Agar media to Lysine Iron Agar and Tryptic Soy Iron for confirmation.

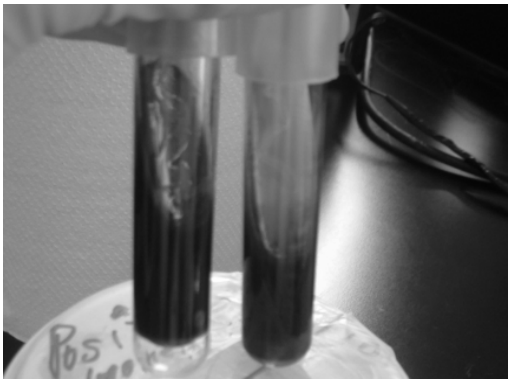

Figure D-5. Positive Lysine Iron Agar and Tryptic Soy Iron slants for *Salmonella*.

Figure D-6. Confirming *Salmonella* colonies with *Salmonella* test kits.

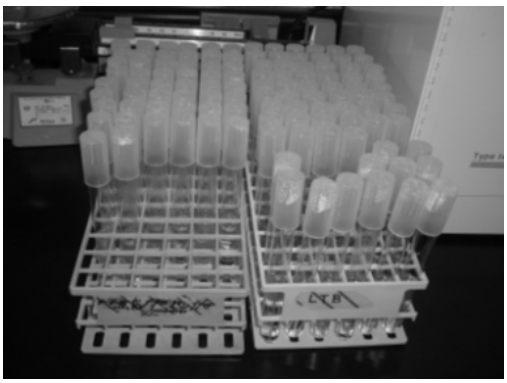

Figure D-7. Lauryl Tryptose broth media for fecal coliforms.

Figure D-8. Lauryl Tryptose broth media in incubator at 35 degree Celsius.

Figure D-9. Sedimentation of samples for the recovery of helminth eggs.

APPENDIX E - PICTURES OF HELMINTH OVA

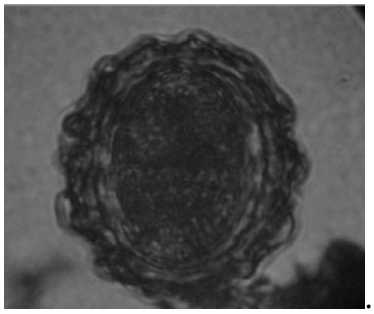

Figure E-1. *Ascaris* egg in sludge from Mexico City wastewater at the Treatment Facility of Texcoco.

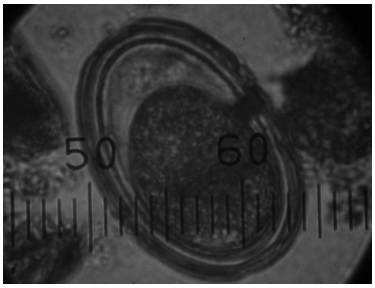

Figure E-2. Decorticated *Ascaris* egg.

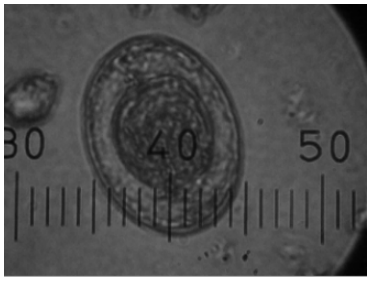

Figure E-3. *Hymenolepis nana.*

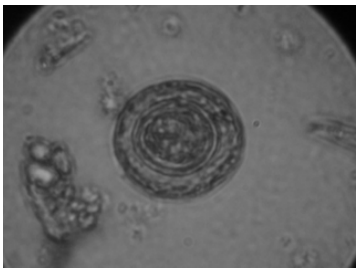

Figure E-4. *Hymenolepis diminuta.*

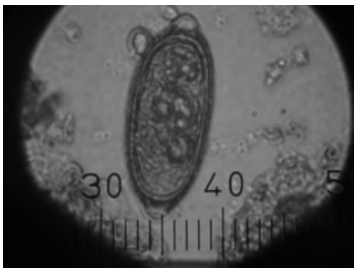

Figure E-5. *Trichuris* spp.

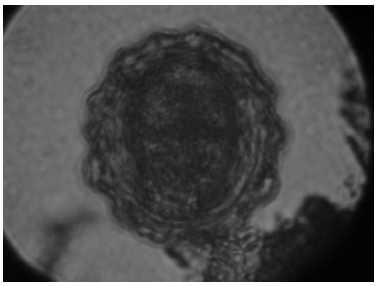

Figure E-6. *Ascaris* egg in sludge from Mexico City.

APPENDIX F - PROTOCOL FOR FECAL COLIFORMS

Materials and media

- 1ml Pipettes
- Test tubes
- Durham tubes (fermentation tubes)
- Lauryl Tryptose Broth (LTB)
- Lactose broth
- EC broth
- Buffered Peptone Water
- Mechanical shaker

1. Add 10g of biosolids to 90ml of buffered peptone water and place on a shaker for at least 10 minutes. This is a 10^{-1} dilution.
2. Make 10-fold dilutions by adding 1ml of the 10^{-1} sample to a test tube containing 9ml of buffered peptone water. Make serial dilution to 10^{-8} or 10^{-9} depending of previous indigenous levels of sample.
3. Add 1ml of each dilution to tubes containing 10ml of either Lauryl Tryptose broth (LTB) or Lactose broth (LB). It is necessary to vortex tubes after dilutions are prepared. Inverted Durham tubes should already be placed inside test tubes. Prepare 3 replicates for each dilution.
4. Incubate samples in a circulating water bath at 35^{o}C for 24 to 48 hours.

5. Score +/- for gas production. There will be air presence in the Durham tubes and the broth will be turbid. Bubbles may also rise in the tube when shaking. These tubes are positive for coliforms.
6. Add 0.1ml from either positive LTB tubes or LB tubes to tubes containing 10ml of EC (*Escherichia Coli*) broth. Do not vortex tubes after inoculating. Inverted Durham tubes should already be placed inside. Incubate in a circulating water bath at 44.5^{o}C for 24 to 48 hours.
7. Score tubes +/- for gas production. These tubes are positives for fecal coliforms.
8. Refer to the MPN table to determine the MPN/g for fecal coliforms.

APPENDIX G - PROTOCOL FOR *SALMONELLA* SPP

Materials and media

- Buffered peptone water
- Rappaport Vassiliadis broth (RV)
- Novobiocin
- Hektoen Enteric
- Tryptic Soy Agar (TSA)
- Lysine Iron Agar (LIA)
- Triple Sugar Iron (TSI)
- Oxoid Latex Test Kit for *Salmonella*
- Petri dishes
- Test tubes
- Pipettes
- Straight and round-ended loops

1. Add 10g for biosolids sample to each one of four bottles containing 10ml of sterile buffered peptone water and shake or stir for at least 10 minutes. Each bottle may be labeled as A, B, C, and D.
2. Add 10 ml of the mixture from bottle D to each one of three empty sterile test tubes. Take 1ml of the mixture of the same bottle D and add to each one of three test tubes containing 9ml of sterile buffered peptone water. Vortex dilution. Discard this bottle after taking the samples. In this step it can be observed that bottles are inoculated with 10g of biosolids, three test tubes with 1g of biosolids, and three test tubes with 0.1g of biosolids. A triplicate dilution and nine solutions are created.

3. Incubate bottles A, B, and C and all test tubes at 35^{o}C for 24 hours in a water bath.
4. Without mixing the dilutions, take 0.1ml of each bottle and test tubes and add it to tubes containing 10ml of Rappaport-Vassiliadis R10 broth. Vortex and incubate at 42^{o}C for 24 hours in a water bath. RV media preparation includes addition of novobiocin, compound that inhibits gram-positive bacteria and increases *Salmonella* population.
5. Take one droplet with an inoculation loop and strike it in Hektoen Agar plates. Incubate at 37 ^{o}C for 24 hours. *Salmonella* colonies grow on Hektoen as separate black and round dots with a colorless halo. Results of possible *Salmonella* colonies should be recorded.
6. Take two typical and separate suspected *Salmonella* colonies and strike them separately in the same Trypticasein Soy Agar (TSA) plate. TSA plates may be half divided for each colony. Incubate for 37^{o}C for 24 hours. In this step, pure colonies are grown in plates.
7. Using a straight needle, pick the center of a well-isolated colony from TSA plates.
8. Inoculate by stabbing to the base of the butt and streaking the slant of LIA and TSI tubes. TSA plates need to be saved for a later confirmation step.
9. Cap the tubes loosely to ensure aerobic conditions. Incubate at 35^{o}C for 18 to 24 hours.
10. For TSI tubes, read for acid production in base of slants which will turn in yellow bases. Also inspect for alkaline slants which will turn in red. Hydrogen sulfide reactions may be present.

11. For LIA, examine at 18 to 24 hours and 40 to 48 hours for alkaline production in the base of the slant (purple color). Blackening at the apex of the slants will reveal hydrogen sulfide productions.
12. Record tubes as positives for S*almonella* spp. if TSI and LIA conditions of *Salmonella* presence are observed. Otherwise, confirmation of results should be made using a latex *Salmonella* test kit.
13. Place one drop of each the reacting and control reagents on a testing card.
14. Transfer the center of a well-isolated colony on the TSA plate to the reacting reagent drop in the testing card using a sterile inoculating loop. Rotate the inoculating loop around the test circle for about 10 to 15 seconds until the circle is entirely covered.
15. Transfer the center of another well-isolated colony on the TSA plate to the control reagent drop in the testing card using a sterile inoculating loop. Rotate the inoculating loop around the test circle for about 10 to 15 seconds until the circle is entirely covered.
16. Rock the card counterclockwise motion for about one to two minutes and inspect for agglutination. A positive confirmation for *Salmonella* spp. is recorded if agglutination occurs in the test reagent (latex test) and does not occur on the control latex sample. If both latex do not present agglutination, the sample is recorded as negative. In the case that both latex agglutinate, the sample should be retested; otherwise results cannot be considered as either positive or negative samples.
17. Based on the number of positive tubes, determine the MPN of *Salmonella*/4g of dry weight biosolids.

APPENDIX H - PROTOCOL FOR HELMINTH OVA

Materials and Reagents

- 2.0 L graduate plastic beakers
- Blender
- Applicators, wooden, 6 inches long
- 160 mesh sieve
- 200 ml centrifuge bottles
- 50 mL centrifuge tubes
- Tween 80
- Acid-alcohol: 0.1N H2SO4
- Ethyl acetate or Diethyl ether
- $ZnSO_4$ (specific gravity 1.3)
- Distilled water

1. Place the equivalent of 2g of dry solids in blender with 200ml of 0.1% Tween 80 and blend for one minute. Recover sample in 2-liter container using 800ml of 0.1% Tween 80 to rinse blender. Settle overnight.
2. Aspirate supernatant avoiding disturbance of sediments. Filter sediment through 160µm-mesh-sieve. Rinse the filtrate with one liter of distilled water into the original container. Settle for at least three hours.
3. Aspirate supernatant. Transfer sediment to a 200-ml centrifuge bottles, making sure of thoroughly rinsing containers. More than one bottle may be used in the process.
4. Centrifuge at 1000 × g for five minutes and aspirate supernatant.

5. Suspend sediment in 150ml of $ZnSO_4$ (specific gravity 1.3). Mix carefully with glass stir rod or plastic spatula. Centrifuge at 1000 × g for five minutes.
6. Recover supernatant in a 2-liter container and add 1-liter of distilled water. Settle for at least three hours.
7. Aspirate supernatant. Transfer sediment to a 200 ml centrifuge bottle. Centrifuge at 1000 × g for five minutes and aspirate supernatant.
8. Transfer sediment to a 50 ml centrifuge tube. Centrifuge at 1000 × g for five minutes and aspirate to 5 ml. More than one centrifuge tube may be used.
9. Add 15 ml of acid-alcohol and 10 ml of diethyl ether. Shake vigorously for two minutes. Allow gas to escape periodically.
10. Centrifuge and aspirate to 5 ml.
11. Add 5 ml of 0.1 N H_2SO_4 for a first washing. Centrifuge at 1000 × g for five minutes.
12. Aspirate supernatant to 5 ml. Add 5 ml of 0.1 N H_2SO_4 for a second washing. Centrifuge at 1000 × g for five minutes. Aspirate supernatant to 5 ml. A third washing may be necessary for samples still with high sediment contents.
13. Examine microscopically to enumerate the detected ova, transferring aliquot to a Sedgwich Rafter cell or Doncaster disk. Distribute aliquots in cell to avoid over position of structures that will difficult the enumeration. Express results in eggs/2g TS in the case for Mexican standard and eggs/4g TS in the case for EPA standards.

REFERENCES

American Public Health Association. 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. APHA, Washington, DC.

Alouini, Z., and M. Jemli. 2001. Destruction of helminth eggs by photosensitized porphyrin. *The Royal Society of Chemistry*. 3:548-551.

Barajas, A. M., and L. Dendooven. 2000. Nitrogen, carbon and phosphorus mineralization in soils from semi-arid highlands of Central Mexico amended with tannery sludge. *Bioresource technology*. Mexico, DF. 77(2001):121-130.

Barrios, J. A., B. Jiménez, and C. Maya. 2004. Treatment of Sludge with Peracetic Acid to reduce the microbial content. *Residuals Science and Technology*. 1(1):69-74.

Barrios, J. A., B. Jiménez, G. Salgado, A Garibay, and A. Castrejon. 2001. Growth of fecal Coliforms and *salmonella spp.* in physicochemical sludge treated with acetic acid. *Water Science and Technology*. 44(10):85-90.

Bowman, D. D., M. D. Little, and R. S. Reimers. 2002. Precision and Accuracy of an Assay for Detecting *Ascaris* Eggs in Various Biosolids Matrices. *Water Research*. 37(2003):2063-2072.

Cabezut, B. O., and A. Sanchez. 1999. Towards an ontology of wastewater treatment plants: the identification phase. *Environmental Modelling and Software*. 14(1999):401-408.

Chavez, A., B. Jimenez, and C. Maya. 2004. Particle size distribution as a useful tool for microbial detection. *Water Science and Technology*. 50(2):179-186.

Chen, G., P. L. Yue, and A. S. Mujumdar. 2002. Sludge dewatering and drying. *Drying Technology*. 20(4&5):883-916.

Choi, C. Y., M. Grabau, S. A. O'Shaughnessy, and I. L. Pepper. 2005. Pathogen Reduction in Biosolids for Land Application. *Journal of Residual Science and Technology*. 2(3):159-171.

De Victorica, J., and M. Galvan. 2002. Preliminary testing of a rapid coupled methodology for quantitation/viability determination of helminth eggs in raw and treated wastewater. *Water Research*. 37(2003):1278-1287.

Escalante, V., G. Moeller, and A. Rivas. 2000. Pond evaluation and upgrading in Mexico. *Water Science & Technology*. Mexican Institute of Water Technology. 42(10): 43–50.

Franco, H. O., A. N. Mckelligan, A. M. Lopez, F. Espinosa, E. Escamilla, and L. Dendooven. 2002. Dynamics of carbon, nitrogen and phosphorus in soil amended with irradiated, pasteurized and limed biosolids. *Bioresource Technology.* 87(2003):93-102.

INEGI. XII Censo General de Población y Vivienda. 2000.

Jimenez, B. 2005. Treatment technology and standards for agricultural wastewater reuse: a case study in Mexico. *Irrigation and Drainage*. 54:S23-S33.

Jimenez, B., J. E. Barrios, J. M. Mendez, and J. Diaz. 2004. Sustainable sludge management in developing countries. Water Science and Technology. 49(10):251-258.

Jimenez, B., and J. E. Barrios. 2001. Environmental Engineering in Mexico. *Journal of professional issues in engineering education and practice*. 127(2):75-83.

Jimenez, B., C. Maya, and G. Salgado. 2001. The elimination of helminth ova, faecal coliforms, salmonella and protozoan cysts by various physicochemical processes in wastewater in sludge. *Water Science and Technology*. 43(12):179-182.

Jimenez, B., J. A. Barrios, and C. Maya. 2000. Class B biosolids production from wastewater sludge with high pathogenic content generated in an advance primary treatment. *Water Science and Technology*. 42(9): 103-110.

Jimenez, B., and V. H. Landa. 1998. Physico-chemical and bacteriological characterization of wastewater from Mexico City. *Water Science and Technology*. 37(1):1-8.

Jimenez, B., and A. Chavez. 1997. Treatment of Mexico City wastewater for irrigation purpose. *Environmental Technology*. 18:721-736.

Mendez, J. M., B. Jimenez, and C. Maya. 2004. Disinfection kinetics of pathogens in physicochemical sludge treated with ammonia. *Water Science and Technology*. 50(9):67-74.

National Water Commission of Mexico. CNA. 2004. Available at: http://www.cna.gob.mx/eCNA/Espaniol/Publicaciones/Subsector2004/tercero.pdf Accessed 5 January 2004.

Nelson, K. L, B. Jimenez, G. Tchobanoglous, and J. L. Darby. 2003. Sludge accumulation, characteristics, and pathogen inactivation in four primary waste stabilization ponds in central Mexico. *Water research*. 38(2004):111-127.

Nelson, K. L. and J. L. Darby. 2001. Inactivation of viable *Ascaris* eggs by Reagents during enumeration. *Applied and Environmental Microbiology*. 67(12):5453-5459.

Nelson, K., and B. Jimenez. 2000. Sludge Accumulation, properties and degradation in a waste stabilization pond in Mexico. *Water Science and Technology*. 42(10-11):231-236.

Norma Oficial Mexicana. NOM-004-SEMARNAT. 2002. Protección ambiental.- Lodos y biosolidos.- Especificaciones y límites máximos permisibles de contaminantes para su aprovechamiento y disposición final. Available at: http://www.irapuato.gob.mx/contenido/uaip/info/leyesyreglamentos/leyes/leyes_ecologicas_japami.pdf. Accessed 15 August 2004.

O'Shaughnessy, S. A., C. Y. Choi, M. Grabau, C. P. Gerba, and I. Pepper. 2005. Reduction of Pathogens in Biosolids in Response to Stress Units using Solar Drying Beds in an Arid/Semi-arid Climate, Real-time Data Acquisition. Water and Environmental Federation Conference for Disinfection.

Peng, W., X. Zhou, and R. B. Gasser. 2003. *Ascaris* eggs profiles in human faeces: biological and epidemiological implications. *Parasitology*. 127:283-290.

Sherman, R. 2002. Snapshots of selected large scale vermicomposting operations. NC State University. Available at: http://www.bae.ncsu.edu/people/faculty/sherman. Accessed 20 September 2004.

Servicio Meteorológico Nacional, Automated weather stations in Mexico. Comisión Nacional del Agua. CNA, 2005.

United Nations Environment Programme. UNEP. 2000. Available at: http://www.unep.or.jp/ietc/ESTdir/Pub/MSW/RO/Latin_A/Topic_d.asp. Accessed 30 September 2004.

U.S. Environmental Protection Agency. 2003. EPA Method 1682: Salmonella in Bio-solids by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium, Draft Document.

U.S. Environmental Protection Agency. 2002. EPA Method 1680: Fecal Coliforms in Bio-solids by Multiple Tube Fermentation Procedures, Draft Document.

U.S. Environmental Protection Agency. EPA. 1994. Environmental Regulations and Technology, Control of Pathogens and Vector Attraction in Sewage Sludge EPA/625/R-92/013.

U.S. Environmental Protection Agency. EPA. 1989. *POTW Sludge* Sampling and Analysis Guidance Document. Document No.:PB93-227957.