Ecological Sanitation: Inactivation of Pathogens in Faeces from Dry Toilet – Greywater Disposal

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Abstract: A project to assess health implications in the implementation of ecological alternatives of sanitation systems in slums was developed in Rosario, Argentina. A settlement, with very poor sanitary conditions, of about 180 families was selected. A dry toilet and a "washwater garden" (greywater disposal) were built in different houses. The objectives were: a) To investigate the inactivation of *Ascaris lumbricoides* eggs, the decay of Fecal Coliforms (Thermotolerant) and the survival of *Salmonella* sp., in faeces collected with soil and lime. b) To characterize greywater and evaluate the functioning of "washwater gardens" under different weather conditions. The "washwater garden" is an appropriate solution for the infiltration of greywater where no other treatment alternative is possible. As a high level of Fecal Coliforms (Thermotolerant) was detected it is recommended to verify the total inactivation of pathogens before its reuse for agricultural purposes or soil infiltration. In the dry toilet, the viability percentage average of *A. lumbricoides* eggs dropped from 86.8% during 80 days of collection to 53.1% during the following 90 days of dehydration but viable eggs count, 39.6 eggs/10g DM in average, remained too high to comply with regulations for unrestricted use of faeces in agriculture. *Salmonella* sp. did not survive at pH 12.5, and no regrowth was detected after 5 days.
Keywords: *Áscaris lumbricoides,* dry toilet, inactivation, *Salmonella* sp., "washwater garden".

INTRODUCTION

A project to assess health implications in the implementation of ecological alternatives of sanitation systems in slums was developed at the Centro de Ingeniería Sanitaria (Sanitary Engineering Center), Faculty of Exact Sciences, Engineering and Surveying, National University of Rosario, Argentina. A settlement of about 180 families located along a stream that crosses the city of Rosario – 300 km northwest of Buenos Aires, capital of Argentina – was selected. Sanitary conditions are very poor: drinking water is supplied from illegal connections made by the neighbours, greywater is disposed of to the ground resulting in stagnant puddles and excreta are collected in cesspools which quite regularly overflow due to the rise of grownwater level during the rainy season. Parasitological analyses were made in fecal samples obtained from 10 children whose ages ranged from 1 to 12 years old. The results indicated the presence of the following parasites: *Ascaris lumbricoides*, *Giardia lamblia, Entamoeba coli* and *Blastocystis hommini*. In order to demonstrate that sanitary conditions could be improved in the slum:

- A dry toilet was built in one house. It should be noted that this house was selected as some members of the family were infected with *Áscaris lumbricoides,* whose eggs are the most resistant to adverse environmental conditions (Feachem, 1983).
- A "washwater garden" was built in each one of two other houses to avoid the direct discharge of greywater on the soil.

OBJECTIVES

• To investigate during the dehydration and composting of faeces: the inactivation of *Ascaris lumbricoides* eggs, the decay of Fecal Coliforms (Thermotolerant) and the survival of *Salmonella*.

• To characterize greywater and evaluate the functioning of "washwater gardens" under different weather conditions.

MATERIAL AND METHODS

Construction and Operation of Ecological Systems

Dry Toilet: In one of the houses in the settlement a dry toilet was built, it was equipped with a urine diverting toilet, urinals and a hand wash sink (Fig. 1).

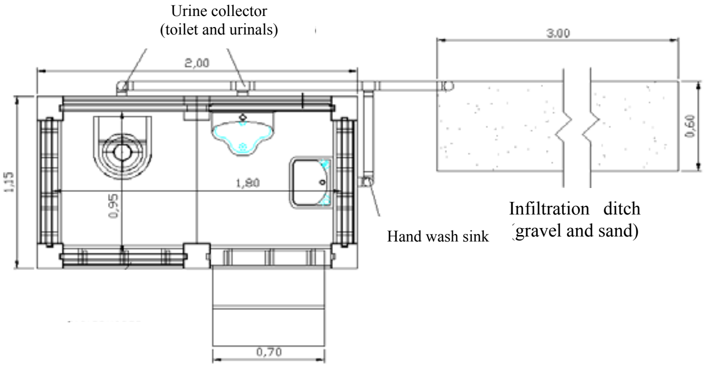


Fig. 1 – Ecological Toilet Design (Layout)

Beneath the floor of the bathroom two chambers were constructed, one of them has a 350-liter plastic container which receives the faeces from the toilet (Collection Chamber), the second is used for the dehydration of faeces (Dehydration Chamber) (Fig 2 and 3). The urine is diverted to an infiltration ditch where drainage, from the urinals and the hand wash sink, is also diverted.

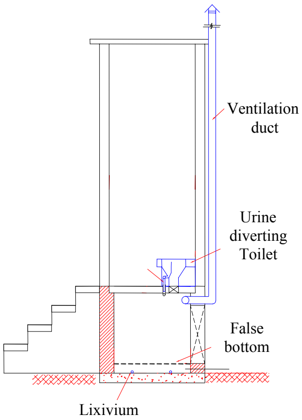


Fig. 2 – Dry Toilet (Cross Section)

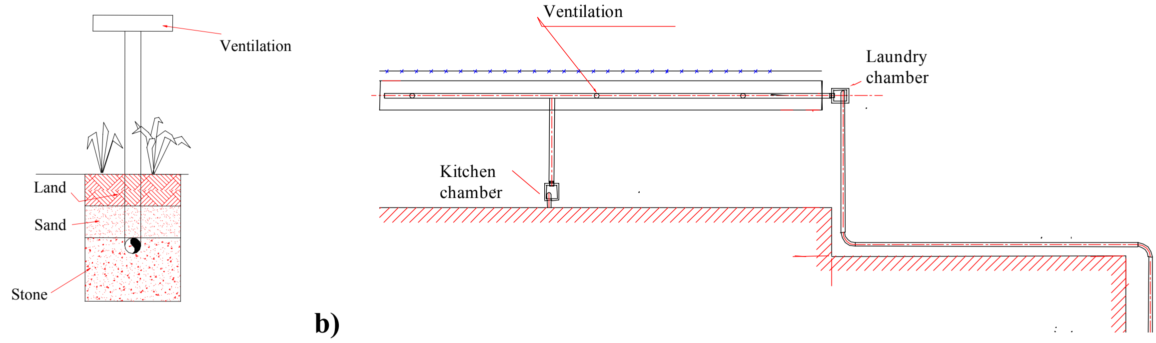
Fig. 3 – Dehydration Chamber

Fig. 4 – Urine Diverting Toilet

Elements of pre-reinforced concrete were used to build the dry toilet. A single vent for both chambers was placed. It was made of a galvanized iron pipe of 150 mm in diameter with a height of 1.50 m above the roof. Since in Argentina there are no diverting urine toilets, one made of stainless steel, fabricated for prisons, was adapted with a screen separator (Fig. 4). The modified toilet was introduced into a concrete pedestal. The dry toilet began operating in April 2007 and was used by about six people. After defecation, a scoop of a dry soil and lime mixture was added, in a 3:1 ratio, respectively. Following three months of use, the plastic container was removed from the collection chamber and placed in the adjacent chamber for the dehydration of the collected material. During the collection period approximately 100 liters of the soil + lime + faeces mixture was accumulated, accounting for a volume of 0.18 liters per person per day.

"Washwater garden": It is a modification of what is formerly known as nitrification ditch or infiltration ditch. It consists of a 0.80 m deep and 0.60 m wide trench where gravel, sand and soil are placed in layers (Fig. 5). A 100 mm diameter perforated pipeline distributes greywater that is infiltrated in the soil. Prior to the entry of greywater in the trench, grease chambers must be placed to eliminate floating and settlement solids (Fig. 6). Ventilation ducts are placed to ensure that the process is aerobic; different species of plants are planted to encourage water evaporation (Fig. 7) (Del Porto and Steinfeld, 2000).

In each of the selected households two chambers were placed: one to degrease the kitchen effluent and the other to act as an interceptor chamber for the solids present in the effluents from laundry, showers, sinks. Both effluents (from the two chambers) go to the perforated pipeline placed into the ditch. At the end a well was digged for fluid overflow that failed to infiltrate (Fig. 5 b).



a) b)

Fig. 5 – Greywater Ditch: a) Cross Section, b) Layout

Fig. 6 – Solid Matter and Grease Intercepting Chamber

Fig. 7 – Greywater Garden

"Washwater garden" was designed based on a flow of 50 L/person.day (each house was inhabited by two adults and two children) and taking into account that the soil is sandy and clayey, an infiltration rate of 60L/m^2/day was adopted (Pacheco Jordao and Arruda Pesoa, 1995). The perforated pipe is 9 m in length whith two ventilation ducts.

Ditches began to function in February 2007 (Fig. 7) and there were no problems even in March and April, months when rainfall was atypical for the area. In Rosario, the annual rainfall rate is 1,000 mm but in March 2007, after five days of continuous rain the rainfall rate was 370 mm. During the operation of the "washwater garden" it was detected that the degreasing chamber, which receives the kitchen wastewater, was not working properly due to grease accumulation, so it has to be cleaned bi-weekly. Samples were collected from the degreasing chamber (kitchen effluent) and from the solid interceptor chamber (bathroom and laundry effluents) of one of the houses for the determination of: pH, BOD, COD, Total and Volatile Solids and Fecal Coliforms (Thermotolerant). Although there was presence of fluid in the pit at the end of the ditch, samples were not analyzed because runoff from rainwater and water from cleaning floors drained into the same pit. The objective to characterize the greywater was to provide data for future projects because there is no information available in Argentina on this type of effluent (greywater).

Physical, Chemical and Microbiological Characterization of Fresh Faeces

1 - Fresh Faeces Added to Soil and Lime (Collection Chamber)

Fresh faeces samples were collected bi-weekly from the container placed in the collection chamber under the toilet floor (Fig. 2). Samples consisted of 5 sub-samples drawn from 5 sites on the surface of the material collected. It should be keep in mind that faeces were mixed with soil and lime (3:1), as after each deposition this mix was added to improve faeces dehydration and prevent odors.

<u>Analyzed Parameters and Analytical Techniques Employed:</u>

1 - Humidity and volatile solids: APHA, AWWA, WEF (1998), Standard Methods for the Examination of Water and Wastewater, 20th edition.
2 - Temperature was measured with digital thermometer in situ.
3 - pH: Potentiometric method.

4 - *Salmonella* sp. in biosolids:
a) Pretreatment of the samples of biosolids: method 1682: "*Salmonella* sp. in Biosolids by Enrichment, Selection and Biochemical Characterization". USEPA, 1998.
b) Isolation and identification of *Salmonella* sp.: APHA, AWWA, WEF (1998), Standard Methods for the Examination of Water and Wastewater - 20th edition, modified by Sanguinetti et al. (2005), to reduce the growth of the indigenous flora that masks the development of *Salmonella*.
5 - Fecal Coliforms (thermotolerant):
a) Pretreatment of the samples of biosolids: Method Appendix F. Control of Pathogens and Vector Attraction in Sewage Sludge. EPA/625/R-92/013- Part 503 - 1999.
b) Quantitative determination of Fecal Coliforms (Thermotolerant): APHA, AWWA, WEF (1998), Standard Methods for the Examination of Water and Wastewater - 20th edition. Method 9221 E.
6 - Helminth eggs: USEPA 1992, protocol modified by Janine Schwartzbrod, University of Nancy, France, for the detection, counting and viability of helminth eggs (Gaspard, Schwartzbrod, 1995).

2 - Faeces in Process of Dehydration (Dehydration Chamber)

After nearly three months of faeces collection the container (I) was removed and placed in the adjacent chamber to continue the dehydration process; previously all material was mixed. The container (I) was replaced by container (II) in the collection chamber under the toilet to continue functioning normally. Samples were collected twice a month from container I. Before collecting the samples all content was homogenized. Each sample consisted of 5 subsamples collected from 5 different sites of the container. During the 90-day dehydration period, the following parameters were analyzed: Humidity, Temperature, pH, Volatile Solids, Fecal Coliforms (Thermotolerant), *Salmonella* sp., *Áscaris lumbricoides* eggs count and viability. The same analytical techniques as described above were applied.

3 - Viability *Ascaris lumbricoides* eggs in faeces incubated at 45 ºC and 55 ºC

After the dehydration period faeces should be sanitized by pathogen inactivation in the organic waste composting. Therefore, composting trials at laboratory scale, using different proportions of dehydrated material from dry toilet with kitchen organic waste, were not successful as temperature could not reach 55 ºC (recommended temperature for pathogen inactivation). So, meanwhile new tests of composting were done, samples of the mix faeces + soil + lime from the dehydration chamber were incubated at two different temperatures, at 45 ºC and at 55 ºC, in thermostatically controlled baths during 18 hours. A sample at room temperature was used as a control of eggs viability.

RESULTS AND DISCUSSION

Characterization of Greywater (Table 1)

Table 1 – Characterization of Kitchen and Laundry Effluents

Parameter	Unit	M1		M2		M3		M4		M5	
		L	C	L	C	L	C	L	C	L	C
pH		6.0	ND	7.5	6.5	8.0	6.5	8.0	6.5	8.0	6.5
BOD	mg/L	1000	ND	950	1500	340	7000	1400	3900	350	1500
COD	mg/L	2600	ND	2200	2600	1050	7500	3800	5600	900	2200
Total Solids	mg/L	463	ND	1500	2700	1600	3100	4000	3600	1200	1200
Volatile Solids	%	34	ND	48	67	31	87	39	75	35	59
Fecal Coliforms (Thermotolerant)	MPN/100ml	$2.3x10^3$	ND	$4.3x10^3$	$9.3x10^3$	$>1.1x10^6$	$7.5x10^3$	$1.1x10^6$	$>1.1x10^6$	---	----

L: laundry effluent; C: kitchen effluent; ND: samples could not be collected because the kitchen chamber was full of grease

As it can be seen the quality of both effluents is highly variable. BOD and COD values are very high, but it must be borne in mind that, in this type of housing, very little water is used because the availability is scarce. As expected, the percentage of volatile suspended solids in the kitchen effluent (67 to 87%) is higher than in the laundry effluents (31 to 48%). The values of Fecal Coliforms (Thermotolerant) are also variable and very high in some samples, as reported by other authors (Ottoson, 2003). The pH in the kitchen effluent was lower than in the laundry effluent, probably due to the bacterial degradation of high concentration of organic matter in the degreasing chamber.

Physical, Chemical and Microbiological Characterization of Fresh Faeces

1 - Fresh Faeces Added to Soil and Lime (Collection Chamber)

Faeces were collected over two and a half months. After the first month, samples of the soil + lime + faeces mixture were collected and analyzed twice a month (Table 2).

Table 2 – Physical, Chemical and Microbiological Characterization of Faeces Added to Soil and Lime

Storage Time Days		Temp (ºC)	pH	H (%)	SV (%)	Fecal Coliform (MPN/g DM)			*A. lumbricoides* Eggs Nº eggs/10 g DM			*Ascaris* Eggs Viability (%)		
From 23/04	From 11/07					Replicate 1	Replicate 2	Media	Replicate 1	Replicate 2	Media	Replicate 1	Replicate 2	Media
Collection Chamber. Faeces Collection Started on 23/04/07														
28	**….**	**16**	**12.4**	**25**	**16**	48	48	**48**	495	437	**466**	85.6	89.3	**87.5**
43	**….**	**12**	**12.3**	**28**	**15**	4.2	32	**18**	65	107	**86**	90.4	91.6	**91.2**
63	**….**	**10**	**12.5**	**46**	**27**	5.5	5.5	**5.5**	……	…..	**......**	…..	…..	**......**
79	**….**	**12.0**	**12.7**	**42**	**21**	5.2	5.2	**5.2**	346	988	**667**	81.3	88.7	**86.8**
Transfer to Dehydration Chamber on 11/07/07														
100	**21**	**12.5**	**10.5**	**28**	**16**	4.2	4.2	**4.2**	11	7	**9**	50.0	20.0	**38.5**
115	**35**	**13.8**	**10.2**	**29**	**14**	131	106	**118**	16	26	**21**	52.2	48.6	**50.0**
134	**54**	**17.4**	**9.0**	**35**	**….**	$1.7x10^3$	$1.7x10^3$	**$1.7x10^3$**	8	11	**9**	50.0	40.0	**45.7**
149	**69**	**15.0**	**8.5**	**31**	**34**	$1.6x10^5$	$1.6x10^5$	**$1.6x10^5$**	131	89	**110**	72.4	69.9	**71.4**
168	**88**	**18.1**	**8.7**	**21**	**14**	$9.5x10^3$	$9.5x10^3$	**$9.5x10^3$**	50	47	**49**	50.0	56.2	**53.1**
189	**101**	**21.4**	**8.9**	**16**	**12**	$1.3X10^5$	$1.3X10^5$	**$1.3X10^5$**	537	255	**396**	39.9	41.1	**40.0**

- The **temperatures** recorded in situ (inside the collection chamber) ranged from 10 ºC and 16 ºC, with an average of 12.5 ºC. Note that the average temperature was 10.5 °C (autumn-winter seasons).
- The **pH** recorded after the first 30 days of faeces collection was around 12.4 and remained approximately in this value over the next 50 days.
- The percentage of **humidity** randomly varied between 25% and 46%, with an average of 35.2%. This variation could be attributed to the fresh faeces received in the collection container during this period. Samples were taken from the surface of the container where faeces had a variable moisture content.
- **Volatile solids** ranged from 15% to 27% with an average of 19.8%, probably for the same reason stated above.
- **Fecal Coliforms (Thermotolerant)** ranged, throughout this period, between 5.2 and 48 MPN/gDM (dry matter); reproducibility of results were obtained in the replicates. Clearly these too low values, not common in the case of fresh faeces, were attributed to the high pH produced by adding lime. The geometric mean in this period was 11 MPN/gDM.
- ***Salmonella*** sp. was not detected in the first 3 samples. This result had been expected not only because the members of the family had no episodes of diarrhea during the study period, but also by the high pH of the samples tested. Nevertheless a test was carried out contaminating the sample of the dry toilet with a suspension of *Salmonella enteritidis* (concentration of approximately 10^9/ml) so as to obtain a final concentration of 10^7/gDM in the sample. Survival of *Salmonella enteritidis* was determined after the following contact times: 1h, 24h, 48h, 5 days. All samples were negative, as expected, as pH > 12. The period of 5 days was selected based on the probability of a *Salmonella* regrowth after the lag phase.
- In relation to the presence of parasites, the faeces of the six members of the family were examined and the results showed that two of them were infected with ***Ascaris lumbricoides***.

Since the eggs of *Ascaris lumbricoides* are highly resistant to different faeces treatments and they are also very persistent in the environment, its inactivation would indicate the elimination of other pathogens. *Ascaris* eggs have therefore become good indicators of the hygienic quality of fecal matter reused as a fertilizer (Feachem, 1983).

• Regarding **eggs count** during the test it should be underscored that samples were analyzed after destroying mechanically formed faeces, nevertheless in the replicates results could not be reproduced. This could be attributed to the random distribution of eggs in a single sample, specially because faeces could not be totally disaggregated and therefore they were not evenly distributed in both replicates. Another reason could be that not all the faeces present in the same sample were from parasitized individuals (only two members of the family were parasitized).

• Besides, **total number of *Ascaris*** eggs was highly variable during the collection period, perhaps for the same reasons stated above. In one of the 5 samples no eggs were found while, in the remaining four, the eggs count varied between 86 and 1230 eggs/10g DM (arithmetic average of replicates), being the overall average of 406 eggs/10g DM (Fig. 8).

• Regarding ***Ascaris lumbricoides* eggs viability**, despite the great variation in the total count, it always remained high, between 86.8% and 91.2% (average of replicates) with an overall average of 88.5% (Fig. 9).

• Eggs inactivation percentage was very low at pH > 12 since the contact time of lime with faeces, at the moment of sample collection, was from hours to 15 - 20 days (time elapsed between two samplings and considering that the samples were taken from the surface of the collected material that represents recent depositions). These results are consistent with what was found by Gaspard et al. (1995) and Strauch et al. (1998) who reported that pH > 12 is required for at least 3 months of fecal storage to inactivate *Ascaris* eggs **in sewage sludge**.

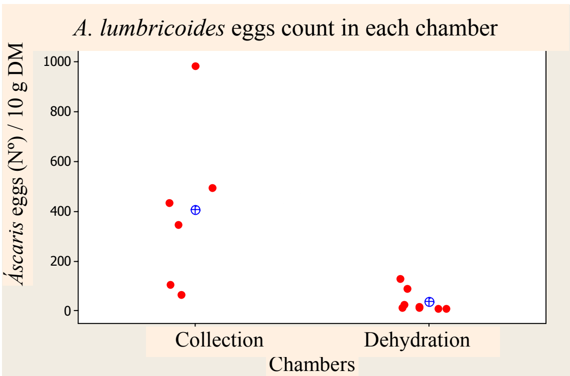


Fig. 8 – Ascaris eggs count (⊗: Average)

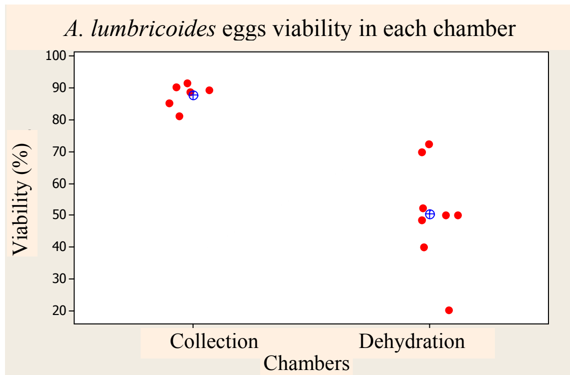


Fig. 9 – Ascaris eggs viability in chambers (⊗: Mean)

Taking into account that in this project **fresh faeces** were analyzed (not sludge from sewage treatment plants), it could be assumed that longer contact times at pH > 12 would be required. It should be noted that the outer membrane of *Ascaris* eggs from sewage sludge may be partially damaged, making them more susceptible to the action of high pH; whereas in fresh faeces eggs membranes could be more resistant to those pH and the larvae could develop without being significantly affected by a pH > 12 for at least a period of 20 days (maximum interval time between two samplings).

2 - Faeces in Process of Drying (Dehydration Chamber) (Table 2)

• The in situ **temperature** of the material accumulated ranged between 12.5 ºC and 21.4 ºC, with an average of 16.4 ºC during 90 days in process of dehydration. The in situ temperatures were 2 °C above the environmental ones for the same day of sampling. The monthly average environmental temperatures ranged between 11 ºC and 20 ºC (winter-spring seasons).

- The **pH** decreased in 20 days, since the beginning of the dehydration process, from 12.7 (collection chamber) to 10.6 and was gradually decreasing up to pH 8.7 over the next 70 days. So, in 90 days the pH decreased by 2 units. The decrease in pH could be attributed to two causes: the disruption of adding lime and the acidity produced by the bacterial metabolism, since after a period of time the few surviving bacteria that have been acclimatized to both very alkaline pH and low humidity, regrew and degraded the organic matter.
- **Humidity** after 90 days of storage in the dehydration chamber varied between 21% and 35% with an average of 27.5.
- **Fecal Coliform (Thermotolerant)** increased with time due to the regrowth produced by the pH decrease and temperature increase. The results were between 4.2 and $1.6x10^5$ MPN/gDM. The geometrical average in this period was $4.2x10^3$ MPN/g DM.
- The **total number of *Ascaris lumbricoides* eggs** (39.6 eggs/10g DM in average) decreased significantly, being the replicates more reproducible in relation with those corresponding to the samples count on the collection chamber. This was mainly due to the dilution that occurred during the thorough mixing of the material in the dehydration chamber and probably also due to the destruction of eggs by the high pH that remained throughout all the storage period.
- **Eggs viability** percentage also decreased and this could be attributed to the samples that contained a mixture of *Ascaris* eggs with different storage times and different pH values (between 12.4 and 8.5). The viability percentage average was 51.7%. It should be noted that pH > 12 was maintained in the material consisting of soil + lime + faeces for 80 days (period of faeces collection) and decreased gradually to pH values between 8.5 and 10.6 for the next 90 days (dehydration period).

It should be noted that after **515 days** of dehydration, two new samples were collected and analyzed. The results were: pH: 7.9; Temperature: 17.3 ºC; Humidity: 7%; Volatile Solids: 6%; **Eggs count: not detected**.

3 - Viability of *Ascaris lumbricoides* eggs in faeces incubated at 45 ºC and 55 ºC

The results of *A. lumbricoides* eggs viability at 45 ºC and 55 ºC verified that viability decreases significantly in relation to the control at ambient temperature, data consistent with the temperatures recommended for pathogens reduction in biosolids. For total eggs inactivation, contact time at 55 ºC should be prolonged (Table 3).

Table 3 – Results of *Ascaris lumbricoides* eggs viability test at 45 ºC and 55 ºC

Temperature	% Viability
45 ºC	13
55 ºC	10
Room (control)	86

Statistical Analysis of Results

Hypothesis tests were applied on the average difference between collection and dehydration chambers over the following parameters: Fecal Coliforms (in decimal logarithm), *Ascaris lumbricoides* eggs count and viability (in percentage). For the analysis of eggs count, the sample of 63 days of storage was not included, as no eggs were detected. In all cases a ***t* test** was used for two independent samples, obtaining as a result, significant differences between the chambers for the three parameters ($p = 0.005$, $p = 0.043$ and p = 0.000, respectively).

CONCLUSIONS

In relation to the "washwater garden", it is concluded that it is an appropriate solution for the infiltration of greywater where there is not enough ground to implement other disposal and treatment alternatives. Nevertheless, as a high level of Fecal Coliforms (Thermotolerant) was detected in greywater it is recommended to verify the total inactivation of pathogens before its reuse for agricultural purposes or soil infiltration.

In relation to fresh faeces alkalized with lime (collected in a dry toilet), it can be concluded the following:

- pH was maintained > 12 during 80 days of collection, then decreased from 12.4 to values between 10.5 and 8.5 during the next 90 days of dehydration.
- The viability percentage average of *Ascaris lumbricoides* eggs dropped from 86.8% during the period of 80 days of collection to 53.1% during the following 90 days of dehydration but the number of viable eggs, with an average of 39.6 eggs/10g DM, remained too high to comply with regulations for unrestricted use of faeces in agriculture.
- *Salmonella* sp. did not survive after 1 hour of contact at a pH 12.5, and no regrowth was detected after 5 days.
- Fecal Coliforms (Thermotolerant) were in an average of 11 MPN/g DM at pH 12.5 for 80 days and regrew during the dehydration period of 90 days, gradually increasing its concentration to an average of $4.2x10^3$ when the pH decreased to 8.5.

Research should continue to determine the percentage of inactivation of *Ascaris lumbricoides* eggs during the composting of storaged faeces naturally dehydrated with organic kitchen waste.

BIBLIOGRAFÍA

1- APHA, AWWA, WEF (1998), *Standard Methods for the Examination of Water and Wastewater* – 20th edition.

2- Del Porto, D and Steinfeld, C. (2000) The Composting Toilet System Book. The Center for Ecological Pollution Prevention (CEPP), Concord, Massachusetts 01742-1330.

3- Feachem R. G., Bradley D. J., Garelick H., Mara, D. D. (1983). *Sanitation and Disease-Health Aspects of Excreta and Wastewater Management*. John Wiley & Sons, Chichester/NewYork.

4- Gaspard, P and Schwartzbrod, J (1995). *Helminth Eggs in Wastewater: Quantification Techniche.* Wat. Sci. Tech. Vol. 31, Nº 5-6, pp 443-446.

5- Gaspard ,P.G. Wiart J. Schwartzbrod,J. , Sludge hygienisation: helmint eggs (Ascaris ova) destruction by lime tratament,Rec Res Dev Microbiol 1, pp77-83 , 1997

6- Ottoson, J, Stenström, T.A., Faecal contamination of greywater and associated microbial risks. Water Research, v.37,p. 645-655, 2003

7- Pacheco Jordao, E.,Arruda Pessoa,C. Tratamento de Esgotos Domésticos, 3ª Edic., ABES, Brasil, 1995

8- Sanguinetti, *G;* C. Tortul, M.C. García, V. Ferrer, A. Mont*angero and* M. Strauss – "Investigating Helminth Eggs and *Salmonella* sp. in Stabilization Ponds Treating Septage". Water Science and Technology – Issue 12, v 51. p. 249-252 – IWA Publishing, 2005.

9- Sanguinetti, G; C. Tortul, M.C. García, V. Ferrer, A. Montangero, D. Koné and M. Strauss – "Isolation of Salmonella sp. in sludge from septage treatment plant" . Water Science and Technology – Issue 12 of volume 51. Pages 239-247– IWA Publishing, 2005.

10- Strauch D. Pathogenic microorganisms in sludge anaerobic digestion and disinfection methods to make sludge usable as a fertilizer, Env.Water Manage. V.1, pp 12-26, 1998

11- USEPA (1998). Environmental Regulations and Technology. Method 1682: Salmonella spp. in Biosolids by Enrichment, Selection and Biochemical Characterization. Office of Water, Washington, DC 20460.

12- USEPA (1999). Environmental Regulations and Technology. *Control of Pathogens and Vector Attraction in Sewage Sludge*, Appendix F - EPA/625/R-92/013 - Office of Research and Development.