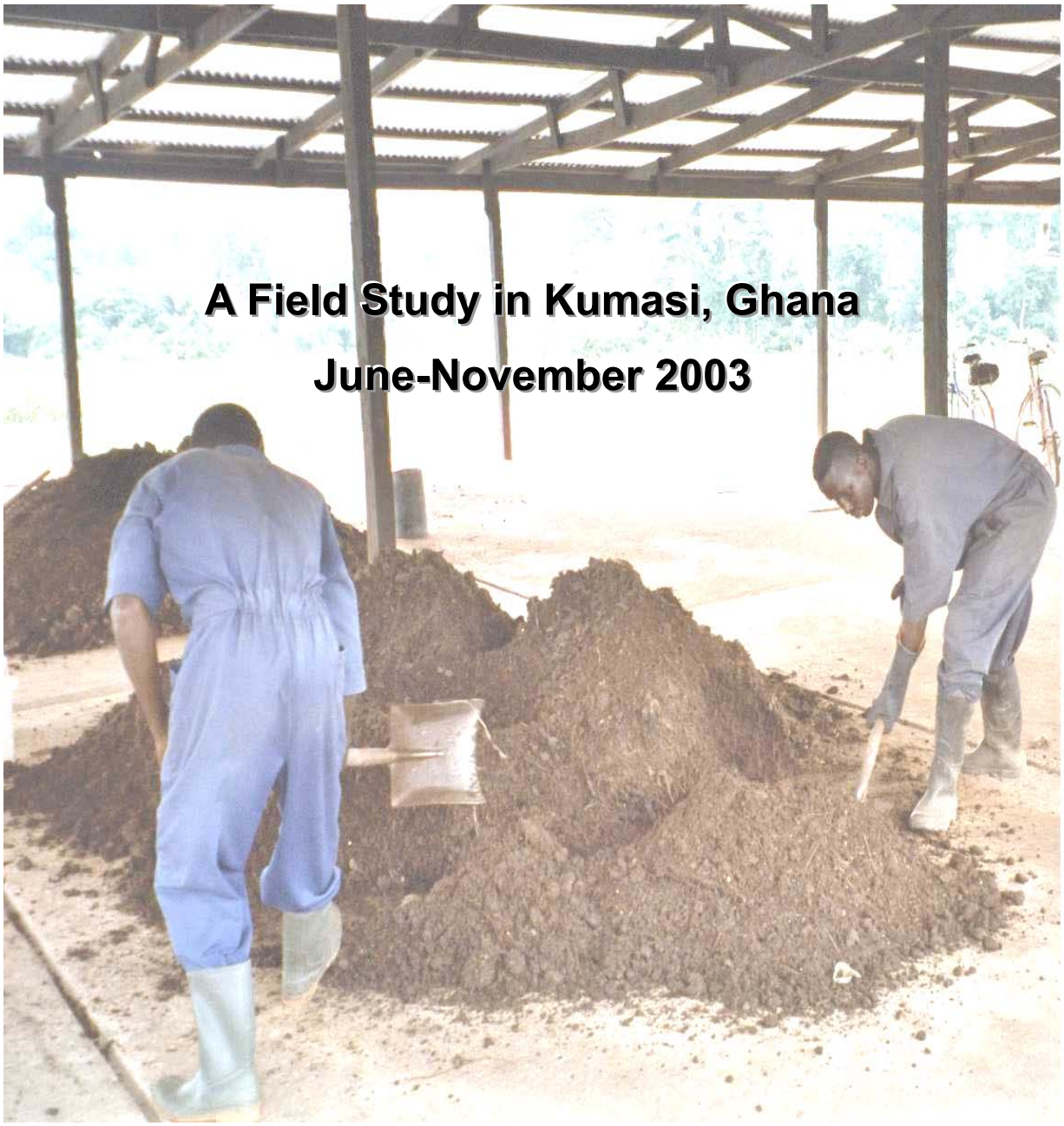
Co-Composting Reduces Helminth Eggs in Fecal Sludge



A Field Study in Kumasi, Ghana

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1 Introduction

1.1 Excreta Reuse in Agriculture

1.1.1 Advantages and Risks

Human excreta are a rich source of organic matter and of inorganic plant nutrients such as nitrogen, phosphorus and potassium. Night soil (i.e. human waste deposited into buckets, pits or vaults) for example contains 10.4-13.1% N_{tot}, 2.7-5.1% P_2O_5 and 2.1-3.5% K_2O in percent of dry matter, or 13g N_{tot}, 4.2g P_2O_5 and 3g K_2O per capita and day (Cross and Strauss, 1985). This would be sufficient to fertilize 600g cereals, i.e. in theory it is nearly sufficient for a person to grow his or her own food (Drangert 1998).

Human excreta are therefore used to support food production in many countries all around the world, especially in China, Southeast Asia and various parts of Africa (Strauss, 2000). However, reuse of untreated feces for agricultural purposes can bare a great health risk, because a great number of pathogens can be found in human excreta. Table 1.1 lists the average number of pathogens expected to be found in one gram of feces in a tropical developing country. This number is in many cases bigger than the median infective dose, which means that already one gram of feces would contain enough pathogens to lead to an infection.

Table 1.1 Average amount of selected pathogens to be expected in feces in a tropical developing country, and the ID_{50} for these pathogens (i.e. the doses required to infect half those exposed). Pathogens for which the median infective dose is lower than the average number of organisms per g feces are shaded (after Feachem et al. 1983).

Pathogen		Average number of organisms per g of feces	Median infective dose (ID_{50})
Viruses	*Enteroviruses*	10^6	low ($<10^2$)
Bacteria	Pathogenic *E.coli*	10^8	high ($>10^6$)
	Salmonella spp.	10^6	high ($>10^6$)
	Vibrio cholerae	10^6	high ($>10^6$)
Protozoa	*Entamoeba histloytica*	$15*10^4$	low ($<10^2$)
Helminth	*Ascaris lumbricoides*	10^4	low ($<10^2$)
	Hoockworms	800	low ($<10^2$)
	Schistosomia mansoni	40	low ($<10^2$)
	Taenia saginata	10^4	low ($<10^2$)
	Trichuris trichiura	$2*10^3$	low ($<10^2$)

1.1.2 Co-Compost: one Option for the Treatment of Fecal Sludge

Thus human excreta provide a good fertilizer, but in order to reduce the health risk for workers, farmers, the near by population and the consumer they have to be treated prior to their use in agriculture. Also, waste water treatment systems usually only remove pathogens from the water.

However, the removed pathogens end up in the biosolids, which still have to be treated in order to be safely used in agriculture (Strauss 2000). Treatment for fecal sludge usually use one or several of the following conditions leading to pathogen die-off: change of pH, UV radiation, chemical treatment, drying, storage for a long time, heat etc (Feachem et al. 1983). Depending on the organism one or the other method is more effective. In the present study, a method was used that combines the effects of heat and time: the fecal sludge was co-composted with organic market waste.

The term co-composting means the composting of two or more raw materials together. In the case of human waste and garbage (the organic part of refuse), this kind of composting is advantageous because the two materials complement each other well. The human waste is high in nitrogen content and moisture and the garbage is high in organic (carbon) content and has good bulking quality. Further more both these waste materials can be converted into a useful product (Obeng and Wright 1987).

Composting can be defined as the biological decomposition of the organic constitutes of wastes under controlled conditions, as a result of the activity of bacteria, fungi, actinomycetes, and protozoa. In the beginning of the process the activity of mesophilic microorganisms (active at temperatures below 40°C) generates heat. When the temperature reaches a certain level, the mesophilic activity begins to decline and thermophilic microorganisms (active at temperatures between 40°C-70°C) take over. This process continues until the temperature becomes limiting for the thermophils, and their population declines. Subsequently the temperature declines and mesophilic organisms increase once again (Obeng and Wright 1987). During the thermophilic phase temperatures of up to 65° or more can be reached.

During such a co-composting process the fecal sludge is sanitized, because the excreted pathogens present in the sludge are destroyed or inactivated during the thermophilic phase. The efficiency of the pathogen inactivation depends on a combination of time and temperature. No of the excreted pathogens can survive a temperature above 65°C for more than a few minutes, however, as soon as the temperatures decrease by only a few degrees, survival increases drastically (Feachem et al. 1983).

Scott investigated Ascaris egg die-off during thermophilic composting already 1952. His stacks consisted of faeces (69 % of raw material), vegetable matter (20 %), soil (10 %), and ash (1 %) and the composting material was turned every 5-10 days. The temperature in the stack at 1 foot depth reached up to 65°C and therefore led to pathogen die-off. Complete helminth egg die-off was achieved within 50 days. Greater than 95 % egg die-off was achieved within 22 day already, though.

1.2 Helminth Eggs as Indicator Organisms

The aim of the present study was to analyze the actual pathogen die-off during the co-composting process. It would be impossible to analyze all pathogen species present in the compost. It was therefore necessary to observe the die-off pattern of an indicator organism that would allow making predictions about the die-off of other pathogens. Of all the pathogen groups, representatives of helminth eggs (before all Ascaris eggs) are the most resistant. They can survive in the environment for many months, and are very resistant to high temperatures (Feachem et al. 1983). It can

therefore be assumed, that if all helminth eggs in the compost are dead, all other pathogens have been removed as well.

1.2.1 Ascaris, Trichuris and Schistosomia

There are many types of helminth eggs that would be suitable as indicator organisms for the pathogen die-off in co-compost. For several reasons it was decided to lay the focus of this study manly on Ascaris and Trichuris eggs. First these two egg species are very common in Sub-Saharan Africa: 173 Mio out of 683 Mio inhabitants are infected with Ascaris, and 162 Mio with Trichuris worms (de Silva et al 2003). Second Ascaris eggs are said to be the most resistant of all excreted pathogens. And third the WHO Guideline for field irrigation with waste water asks for an average of less than 1 Ascaris, Trichuris or Hookworm egg per liter during irrigation period (WHO 1989). It would therefore be of advantage to know the concentration of Ascaris, Trichuris and Hookworms eggs in the final product. However, since Hookworms die off very fast in the environment (100% removal after 24h at temperatures above 35°C, Obeng and Wright, 1987), and because they are quite difficult to identify they were not considered for this study. Instead, the amount of *Schistosomia mansoni* in the compost was determined, because like hookworms they are representatives of the group of helminth eggs that die off very fast, but they are easier to identify than the hookworms (*Schistosomia mansoni* eggs are quite big and have a very characteristic sting-like appendix, see Figure 1.1).

Figure 1.1 ***Schistosomia mansoni (source: WHO 1996)***

1.3 Theoretical Relation between Temperature and Helminth Egg Die-off

As stated before, helminth eggs die during the co-composting process mainly because of the heat that is generated inside of the composting heaps. It is therefore important to know how high these temperatures have to be in order to kill the pathogens. Through an extended literature review Feachem et al. (1983) came up with a theoretical time-temperature relationship leading to the die-off of excreted pathogens. Viennerås et al. (2003) derived equations from these data for the time (t) in hours required to attain no viable organisms at different temperatures (T). For Ascaris and Schistosomia they are as follows (no data exist for Trichuris):

Ascaris: $t = 177 * 10^{-0.1922(T-45)}$

Schistosomia: $t = 10.0 * 10^{-0.1844(T-45)}$

With the help of these equations it can be calculated how long the eggs have to be exposed to a certain temperature in order to die-off. For example inactivation of all Ascaris eggs shloul take place, if the temperature of the compost heaps exceeds 45°C for at least 5 days (see Figure 1.2). The same result could be achieved with 8 days at 44°C, 12 days at 43°C, 19 days at 42°C, one month at 41°C or 1.5 month at 40°C. Schistosomia are much less resistant: they need only 10h at 45°C, 1 day at 43°C and 3.5 days at 40°C to be completely inactivated.

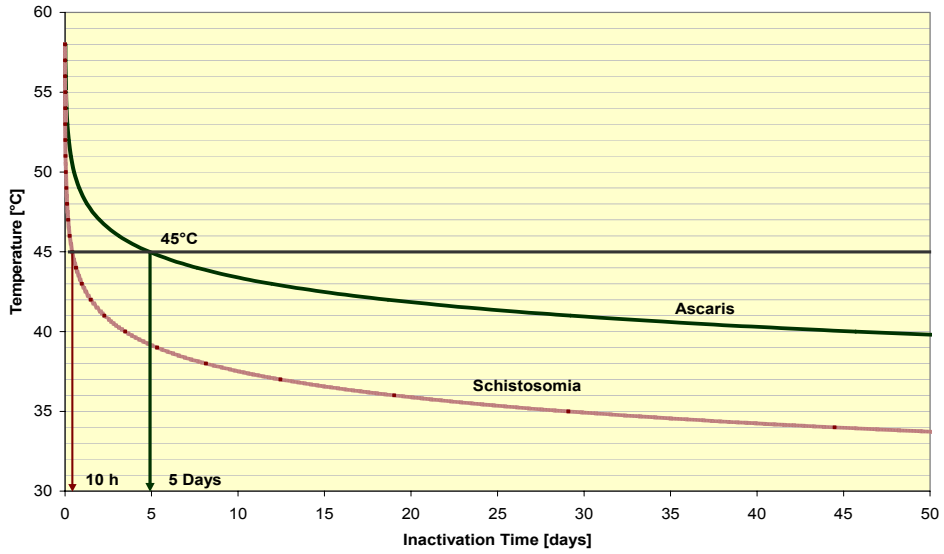


Figure 1.2 Theoretical inactivation times (t) relative to the temperature (T) for Ascaris ($t = 177 * 10^{-0.1922(T-45)}$) and Schistosomia ($t = 10.0 * 10^{-0.1844(T-45)}$). As indicated by the grey lines at a temperature of 45°C Schistosomia would die off after 10h and Ascaris after 5 days. (after Vinneras et al 2002).

1.4 The Aim of this Study

The temperature vs. time die-off curves for pathogens discussed in the previous chapter are very theoretical and derived from literature data. In the present study we will try to analyze the “real-life” process of the helminth egg die-off in co-compost. Data were collected in two different systems: co-compost produced in a pilot plant in Kumasi and composted settled sludge from the composting plant in Teshie (both Ghana). The main questions of interest were:

- ➔ How high is the helminth egg contamination in fecal sludge (i.e. the starting matereal of the composting process) in Kumasi?
- ➔ Do all helminth eggs die-off during the composting process?
- ➔ Is there a relationship between the rate of the helminth egg die-off and the temperatures reached during the composting process?
- ➔ Does the turning frequency of the compost heaps influence the helminth egg die-off?
- ➔ Do helminth eggs that are located inside of the composting heap die-off faster than the ones on the surface of the heap (or vice versa)?

2 Material and Methods

2.1 Location

The co-composting plant was located in Buobai, a neighborhood of Kumasi, Ghana (6°40'N and 1°35' W). All the laboratory examinations were carried out in the Environmental Sanitation Lab of the Civil Engineering Department at Kwame Nkruma University of Science and Technology (KNUST), Kumasi.

2.2 Co-composting Dewatered Fecal Sludge and Market Waste

2.2.1 Fresh Sludge

The fresh fecal sludges used in this study were sludges of variable consistency collected from so-called on-site sanitation systems; viz. latrines, non-sewered public toilets, septic tanks, and aqua privies.

Two types of fecal sludges served as raw material for the dewatering: Septage i.e. contents of septic tanks (usually comprising settled and floating solids as well as the liquid portion), and public toilet sludge collected from unsewered public toilets (usually of higher consistency than septage and biochemically less stabilised).

2.2.2 Dewatered Biosolids

Fecal sludge (FS) from public toilets and septic tanks was mixed in a volume ratio of 1:2 (total volume ca 15m^3, total solid content 2-3%) and loaded onto two drying beds. The beds consisted of a concrete basin with a surface area of 25m^2 filled with different layers of gravel and sand. The top layer consisting of sand acted as a solid-liquid separator by retaining the solid part. The liquid fraction was percolating through the sand and collected by a drainage system at the bottom of the basin. The percolate was collected in another basin for volume determination and sampling. The drying process was stopped as soon as the total solids (TS) content of the sludge was higher that 20%. The dried sludge was then shoveled from the sand and sent for composting. (For more details on the drying bed, refer to IWMI et al. 2003)

2.2.3 Organic market waste

Market waste (MW) was collected on Kumasi Central Market - Aboabo Station in containers provided by the Kumasi Metropolitan Assembly (KMA). It was then brought to the composting plant and hand sorted into its organic and non-organic fractions. The organic fraction was used for composting.

2.2.4 Co-Composting Process

The composting plant consisted of a platform equipped with a drainage system and covered by a roof (see also IWMI et al. 2003). Between June and November 2003 two composting cycles were monitored, Cycle 5 from June 29th to October 15th 2003, and Cycle 6 from August 23rd to November

10th 2003. For each cycle, two compost heaps of $3m^3$ each were formed using $1m^3$ of dried fecal sludge and $2m^3$ of organic market waste. The material was thoroughly mixed and watered if necessary before the heaps were formed.

Every day the temperature of the heap was measured at different locations inside and outside of the heap, using a bimetal thermometer. The inside temperature was determined as the mean of all temperatures measured inside, and the outside temperature was determined as the mean of all temperatures measured on the surface of the heap.

The compost was aerated by turning the heaps inside out. In order to measure the importance of the turning frequency on the performance of the composting process, the heaps were turned with different frequencies. One of the heaps (Heap 1) was turned according to temperature. This means that during the thermophilic phase (inside temperatures higher than 55°) the heap was turned 3 times per week, and afterwards it was turned once per week. The other heap (Heap 2) was turned every 10 days, regardless of the temperature or any other factor. During the turning, the moisture content of the compost was checked in the following manner: A fist-full of compost is taken in the hand and squeezed tightly. If moisture but not free water appears between the fingers, the moisture is ideal; if however, water flows out of the tightly clenched fist, it is too wet (Bakx, 2002). If the material was too dry, water was sprinkled over the compost.

The “active” composting process lasted for about 60 days. During this period the heaps were turned and watered. Afterwards the maturation phase started: the heaps were left to mature without watering or turning. This phase lasted between 3 (Cycle 6) to 6 (Cycle 5) weeks. At the end of the maturation phase the compost was sieved and the final product was bagged.

2.2.5 Sampling

Fresh Sludge: Before the fresh sludge was loaded onto the drying beds, it was filled into a concrete basin and mixed thoroughly. About 2l of sample was taken after mixing.

Compost: During each turning two samples were taken; one from the inside (I) of the heap and one from the outside (O) of the heap. For each sample material from different locations inside or outside of the heap was taken and thoroughly mixed with a shovel. About 2l of this mixture was filled into a polyethylene bag to make the I or the O sample. During the maturation phase no samples were taken. Only the final product was analyzed before and after sieving.

All samples were immediately transported to the lab (about 1 hour away from the plant) and then stored in the fridge until analyzed.

2.3 Composting Settled Sludge and Sawdust

2.3.1 Teshie Composting Plant

A second set of experiments was conducted on the composting plant in Teshie near Accra (5.57° N, 0.11°E). On this plant the facilities for the treatment of fecal sludge tow settling-thickening tanks for solids-liquid separation followed by a series of 3 ponds to treat the fecal sludge supernatant. There are two sedimentation tanks that are used alternatively. When one is being used, the other, which is already full, is allowed sometime for sedimentation to take place after which the pond is desludged.

The plant has currently no functioning machinery for desludging of the ponds. Desludgement is therefore done as and when equipments are available. Desludging the ponds take place concurrently with mixing up the sediment with soft wood saw dust, which is mostly acquired from a timber market sited close to the plant. It is estimated that about 21m^3 of saw dust is mixed with one full pond of sediments which is about 70 tons each time the pond is desludged.

2.3.2 Composting Procedure

After desludging the sedimentation pond, 4 compost heaps (of almost the same volume though not measured) were formed out of a portion of the mixture of settled sludge and sawdust. Two of the heaps were made outside (O H1 and O H2) and two under a shade (S H1 and S H2). Every two weeks the temperature at different locations within the heap was measured, to give the average overall heap temperature. The heaps were turned upside down once per month.

2.3.3 Sampling

During each turning material from different locations within the heap was taken and thoroughly mixed with a shovel. About 2l of this mixture was filled into a polyethylene bag for analyses. The samples were transported to Kumasi as fast as possible (about 4 hours car drive) and then stored in the fridge until analyzed.

2.4 Laboratory Analyzes

2.4.1 Total Solids

About 60g of each sample was weighed into a Petri dish (m_{before}) and then dried for 24h at 105°C. Thereafter the sample was weighted again (m_{after}), and the % of total solids was determined as:
$TS= m_{after} / m_{before}$

2.4.2 Helminth Egg Concentration

For each sample the concentration of Ascaris and Trichuris eggs was determined. The protocol based on the US EPA Protocol (1999) was developed by Schwartzbrod et al. (2003) and slightly modified for the laboratory conditions found in Ghana (for a step by step protocol, please refer to Appendix 7.1):

In general whenever a container was emptied into another, the equipment was rinsed with phosphate buffered H_2O, containing 200mg of OMO washing powder per liter, to prevent the loss of helminth eggs during the process.

250ml of tap water were added to a sample that corresponds to 4g of dry matter (i.e. 4g/TS) and the mixture was blended with a kitchen blender for 1min at high speed. The blended sample was poured into a bucket (size at least 1l), filled up with phosphate buffered H_2O, containing 200mg of OMO per liter to nearly 1 liter and let to soak for at least 3h. These steps should help to separate the eggs from the solid matter.

Then the sample was poured though a sieve (80 mesh) and thoroughly rinsed with tap water. The percolate and the rinsing were collected in a 2l bucked and were let to settle for at least 3h.

After the settling of the eggs the supernatant was aspirated with a water jet pump and the sediment centrifuged in two 150ml tubes for 5min at 400G. The supernatant was poured off and 60ml of $MgSO_4$ solution (specific gravity = 1.29) were added to the pellet in each of the tubes. The pellet was re-suspend by stirring carefully. Because the helminth eggs have a density of about 1.1 (e.g. fertile Ascaris d=1.110 (Thevenot-Lallement, 1984)) they will float in the $MgSO_4$ solution. After waiting for 10min the tubes were centrifuged again for 5min at 800G (brake rate = 0), the supernatant was poured into 2l of tap water, and let to settle for at least 3 hours.

After the settling the supernatant was aspirated using a water jet pump and the sediment was collected in several 15ml tubes and centrifuged for 5 minutes at 800G. If more than 2 tubes were used in the first step, the sediments of all tubes were regrouped in 2 tubes and the centrifugation was repeated. Then the supernatant was poured off and 7ml of H_2SO_4-ethylalcohol and 3ml of ethyl acetate were added to each of the tubes. The tubes were inverted several times and centrifuged for 5 minutes at 660G. With the help of a pipette the two layers of the supernatant were carefully removed.

If no viability test was performed (Cycle 5) the sediment was diluted with 0.1N H_2SO_4 until it was clear enough to be observed under the microscope. The volume of the final sample was noted and with the help of a pipette 1ml of the solution was added to a Sedgwick-Rafter cell. The total number of Ascaris and Trichuris and *Schistosomia mansoni* eggs in the cell was counted with the help of a microscope.

2.4.3 Viability

Viability of the Ascaris Eggs was usually determined with the help of the Safranine dying method developed by de Victorica and Galván (2003): After the last centrifugation (660G) and the removal of the supernatant, the sample was stained by adding 2 to 3 drops of Safranine O (2.5% in H_2O) to the sediment. After waiting for at least 10 minutes the tubes were filled with water and centrifuged for 5 minutes at 800g. The supernatant was poured off, the pellet re-suspended with water, and the tubes centrifuged again. This process was repeated 3 times. The sediment was then diluted with 0.1N H_2SO_4 and the total eggs were counted in the Sedgwick-Rafter cell, as described above. If the dye had penetrated into the Ascaris eggs they were counted non-viable.

However the detection of the viability was not without problems, sometimes it was extremely hard to see, whether the Safranine had really penetrated into the egg, or whether it was just sticking to the outer shell, making the egg look colored. Also, in some of the cases the eggs were imbedded in plant fibers which could have prevented an effective dyeing. Because of these problems, 4 samples were also treated with another viability determining method (incubation method): Instead of dying the sample with Safranine it was incubated for 24 days at 37°C. If afterwards a larva could be detected inside of the egg, it was counted viable. However, also this method had its problems, because it was not always easy to see whether there was really a larva inside of the egg.

2.4.4 Controls

Blank: It is necessary to be sure that the equipment used to prepare the samples was not being contaminated during earlier examinations. For example if a sample with a large number of eggs was prepared and some of these eggs would stay in the equipment, they could later contaminate other samples and thus increase the amount of eggs found there. To be sure this was not the case

some of the equipment (in particular the Sedgwick rafter cell, test tubes, and pipettes), were examined under the microscope, to see whether any eggs were sticking to them. In all of these examinations the equipment was free of helminth eggs.

However, not all of the equipment can be analyzed under the microscope. Therefore a blank sample (garden soil, assumed to be free of helminth eggs) was treated exactly the same way as all the other samples and then the number of helminth eggs was determined.

No helminth eggs were found in the garden soil sample (blank). It was therefore assumed, that no eggs from previously treated samples "contaminate" later treated samples.

Double Counts: In order to test the reproducibility of the results, several samples were prepared and counted twice, in order to see whether the same results would be obtained. However, because the sample preparation is very time-consuming this could only be done in special cases. In other cases the already prepared sample was counted twice, i.e. the number of eggs in two 1ml aliquots of the same purified sample was determined.

Table 2.1 shows the results of these double investigations. It can be seen that the for the Ascaris eggs difference between the two investigations is usually quite small (below 6% in 4 cases, 30% in one case and 50% for the compost sample). This is, however, not always the case for the Trichuris eggs. There the variation between the two investigations can be quite big (up to 100% difference). This could be due to the fact that Trichuris eggs are smaller than the Ascaris eggs and therefore the chance of overlooking them is higher.

Table 2.1 Comparison of the outcome of two investigations of the same sample. In 2 cases the purifying and the counting step were repeated, and in the others only the counting step was repeated.

Sample	1st Investigation			2nd Investigation		
	Ascaris Eggs /gTS	Trichuris Eggs/gTS	Total Eggs/gTS	Ascaris Eggs/gTS	Trichuris Eggs/gTS	Total Eggs/gTS
Samples that were purified and counted twice						
Fresh Sludge Cycle 6	155	110	265	145	73	218
Dewatered Sludge Cycle 7	30	0	30	32	3	35
Samples that were only counted twice						
Dewatered Sludge Cycle 6	40	20	60	38	45	83
Septic 1	30	0	30	30	10	40
PTS 5	12	6	18	18	0	18
Compost	2	0	2	4	0	4

2.4.5 Comparison between Helminth Egg Analysis in Kumasi and Nancy

Four fresh and one dewatered sludge samples analyzed in Kumasi were at the end of the study (after 1 to 3 month storage in the fridge) transported to Europe, and analyzed again in the

Laboratory of Prof. J Schwartzbrod at the Université Henri Poincaré, Nancy. Unfortunately it was not possible to cool the samples during the transport, which means that for one week they were kept at ambient temperature.

Table 7.2 in the Appendix. It turns out, that despite of the long storage and the rough transport the results obtained by the two laboratories are in a similar range. The total number of helminth eggs per g TS found in the two tests vary by a factor 0.46 to 2.33. The viability found in Ghana is, however, always higher. One reason for this could be the long storage and the transport prior to the analyses in Nancy. But also, as mentioned above, the viability tests performed in Ghana were not without problems. It can thus be concluded that the percentage of viable eggs found in this study presents an upper limit, and is probably rather a bit too high.

2.5 Statistical Analyzes

For the statistical analyzes a confidence level was defined as $\alpha = 0.05$.

2.5.1 Comparing Paired Data-Sets: Wilcoxon Matched-Pairs Signed-Ranks Test

The Wilcoxon Matched-Pairs Signed-Ranks Test (Institute of Phonetic Sciences, 2003) was used to determine, whether there is a difference between paired samples. *H0:* The difference (d = x - y) between the members of each pair (x, y) has *median* value zero, i.e. x and y have identical distributions.

The following approximation was used to determine the level of significance:

$$Z = (W - 0.5 - N * (N + 1) / 4) / \sqrt{(N * (N + 1) * (2 * N + 1) / 24)}$$

Where as N = Number of pairs

W = the larger of W+ (sum of all positive ranks) and W- (sum of all negative ranks)

Z has an approximate Standard Normal distribution and tables for the Normal distribution can be used to determine the level of significance (p).

Comparing the worm counts from different heaps can be difficult, because the samples were not always taken at the same time relative to the starting of the composting process. If this was the case, the number of eggs was extrapolated between the samples taken before and after the day of interest (e.g. estimation for [sample day 10] = ([sample day 8]+[sample day 13]) / 2)

2.5.2 Functions to Describe the Helminth Egg Die-Off: Method of Least Squares

For certain investigations it is necessary ti find a function that describes the course of the helminth egg die-off in the compost. It was thus tired to find the best fitting die-off curve. Using the method of the least squares a linear, exponential and quadratic function was fitted to the data. The function with the highest value for R^2 was assumed to best describe the actual die-off.

3 Results

3.1 Helminth Egg Concentrations in Sludges

3.1.1 Fresh Sludge

The helminth egg concentration in several fresh sludge samples coming from either public toilets, septic tanks or a mixture of the two was analyzed. All samples were collected in Kumasi and con thus help to estimate the level of helminth egg contamination in future starting materials used in the Kumasi co-composting plant. The results are summarized in the following table:

Table 3.1 Helminth egg concentration and total solids in several fresh sludge samples collected in Kumasi

Sample Source	Com-post Cycle	% Total Solids (TS)	Number of eggs /g TS			Total helminth eggs /gTS	Total helminth eggs/g Sludge
			Ascaris	**Trichuris**	**Schistos-omia**		
Public Toilet 1		5.6%	83	0	0	83	5
Public Toilet 2		5.5%	15	3	0	18	1
Public Toilet 3		1.7%	114	42	6	162	3
Public Toilet 4		4.5%	80	20	0	100	5
Septic Tank		2.8%	30	5	0	35	1
Public Toilet/ Septic Tank (1:2)	6	2.8%	150	92	0	242	7
Public Toilet / Septic Tank (2:1)	7	2.6%	32	3	0	35	1

The results in Table 3.1 show that all samples analyzed are infected with helminth eggs, but there is a large variation in the degree of infection for the different sludge samples (18 to 242 eggs/gTS). There is no obvious connection between the type of sample source (public toilet or septic tank) and the degree of contamination.

Out of the three helminth egg species analyzed (*Ascaris lumbricoides, Trichuris trichiura,* and *Schistosomia mansoni*) Ascaris is always the most frequent, followed by Trichuris and Schistosomia. This is not surprising, because female *A. lumbricoides* worms produce 200'000 eggs per day, compared to the 2'000-10'000 produced by *T. trichiura*, and only some hundreds produced by *S. mansoni* (Feachem et al. 1983). Also, in Ghana the estimated Trichuris prevalence is much lower than the infection rate for Ascaris: 6.4% and 15.9% respectively (Hotez P (in prep.)).

However, in all cases the helminth egg concentration is well above the recommended value for materials used in agriculture (Xanthoulis and Strauss (1991) proposed a nematode egg standard of ≤ 3-8 eggs/gTS). The sludge will therefore have to be treated before it can be used for agricultural purposes.

3.1.2 Dewatered Sludge

The process of dewatering the fresh sludge on the drying beds could alter the helminth egg concentration per TS: UV radiation and dewatering/drying could cause the eggs to die-off, eggs could be drained out with the percolate, or remain on the drying bed after desludgeing. Therefore the helminth egg concentration of the dried sludge was analyzed as well (see Table 3.2). The helminth egg concentration in the dried sludge ranges from 22 to 83 eggs per g total solids.

Table 3.2 Helminth egg concentration of the dried sludge used for composting

Sample Source	Com-post Cycle	% Total Solids (TS)	Number of eggs/g TS			Total helminth eggs/g TS	Total helminth eggs/g Sludge
			Ascaris	**Trichuris**	**Schistosomia**		
Public Toilet / Septic Tank (1:2)	5	53.6%	23	2	1	26	13
Public Toilet / Septic Tank (1:2)	6	58.9%	38	45	0	83	49
Public Toilet / Septic Tank (2:1)	7	28.4%	16	6	0	22	6

For Cycle 6 and Cycle 7 the sludge was analyzed before (Table 3.1) and after drying (Table 3.2). The comparison of the results given in Table 3.1and Table 3.2 shows that for the two cases analyzed the helminth egg concentration per gram total solids in the dried sludge is lower than in the fresh sludge. However, the helminth egg contamination of the dried sludge still exceeds the 3-8 eggs/g TS recommended for biosolids used in agriculture (Xanthoulis and Strauss, 1991). Therefore the dried sludge has to be hygenized before it can be safely used as fertilizer.

Table 3.2 reveals that there is a large variation in the total solids content of the dried sludges. The reason for that could be that the sludge in cycle 5 and cycle 6 was stored one month and two weeks respectively prior to composting. On the other hand, for the sludge used for Cycle 7 drying was not complete due to technical problems (heavy rain, bad drying-bed performance).

3.1.3 Viability of Ascaris Eggs in Fresh and Dried Sludge

In order to understand the infection potential of the biosolids, not only the absolute eggs counts are important, but also the viability of the eggs. Only viable eggs can be infectious and thus present a health risk. In sludge stored for a long time (e.g. for several years in septic tanks) one may expect a greatly reduced viability. Therefore the percentage of viable Ascaris eggs was determined for some of the fresh and dried sludge samples (see: Table 3.3). The viability of Ascaris in fresh sludge ranges from 40% to 82%, in dried sludge it ranges form 50% to 57%. In most of the cases, more than half of the Ascaris eggs are still alive and infective. Thus, in all of the examined biosolids, a

large number of viable helminth eggs can be found. It is therefore absolutely necessary to treat these biosolids prior to use.

Table 3.3 The viability of Ascaris eggs in fresh and dried sludge. The sample labeling corresponds to Table 3.1 and Table 3.2.

Sample	Total Ascaris [eggs/gTS]	Viable Ascaris [eggs/gTS]	% Viable Ascaris
Public Toilet 1	83	68	82%
Public Toilet 2	15	6	40%
Public Toilet 3	114	78	68%
Septic Tank	30	15	50%
Public Toilet / Septic Tank (1:2)	150	72	48%
Public Toilet / Septic Tank (2:1)	32	24	75%
Dried Sludge (Public Toilet / Septic Tank (1:2))	38	22	57%
Dried Sludge (Public Toilet / Septic Tank (2:1))	16	8	50%

3.2 Co-Compost

3.2.1 Compost Temperatures

The inactivation of the helminth eggs in the compost can be accomplished, if the temperature inside of the heaps is sufficient (Scott 1953, Vinneras et al 2002). As shown in Figure 1.2 it can be said, that theoretical inactivation of all Ascaris eggs will take place, if the temperature of the compost heaps exceeds 45°C for at least 5 days. The same result could be achieved with 8 days at 44°C, 12 days at 43°C, 19 days at 42°C, one month at 41°C or 1.5 month at 40°C.

Figure 3.1and Figure 3.2 show that the inside temperatures of Cycle 6 are in general to be a bit higher than the temperatures of Cycle 5. The mean temperatures over the first 60 days of composting for Cycle 6 for heap 1 (turned according to temperature) and heap 2 (turned every 10 days) are 47.5°C (n=100 SD= 10.4) and 49.8°C (n=100, SD=11.4) respectively, compared to 43.5°C (n=122 SD=11.5) and 44.0°C (n=126 SD=10.6) in Cycle 5. However, there is no obvious difference between the turning regimes within the same cycle.

For all heaps in both cycles the temperatures inside and outside are above 45°C for more than 4 days (grey line in Figure 3.1 and Figure 3.2). This means, that the temperature theoretically necessary to ensure the die-off of Ascaris eggs and other pathogens is reached in all cases. However, in this study the actual concentration of helminth eggs in the compost will still be monitored to verify the theoretical assumptions.

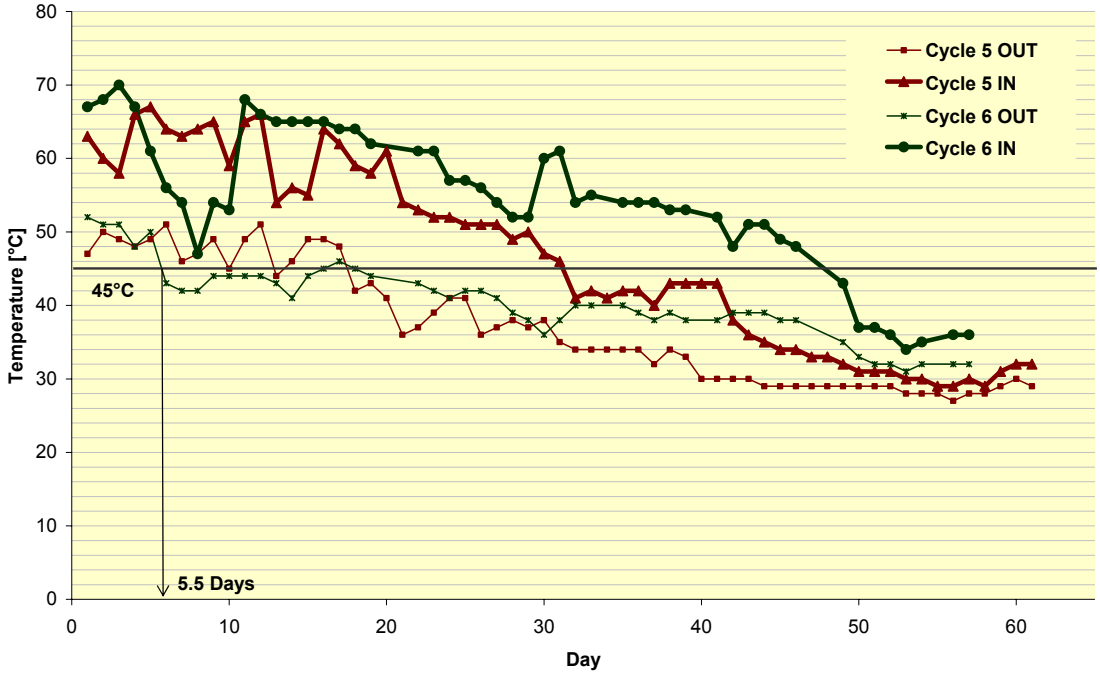


Figure 3.1 Temperature progression for Cycle 5 and Cycle 6 inside and outside of the heaps turned according to temperature.

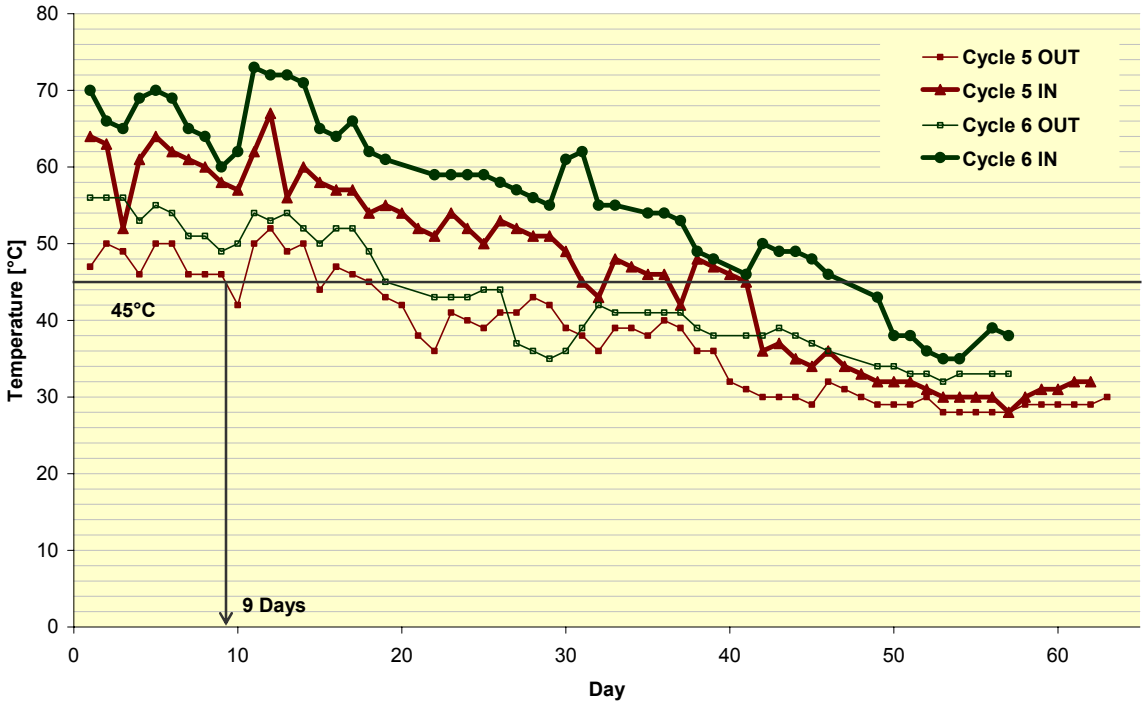


Figure 3.2 Temperature progression for Cycle 5 and Cycle 6 inside and outside of the heaps turned every 10 days.

3.2.2 Influence of the Location within the Heap

This chapter examines whether the location within the heap influences the die-off rate of helminth eggs, i.e. whether the eggs inside die faster than the ones outside or vice versa. Figure 3.3, Figure 3.4 and Figure 7.1, Figure 7.2 of the appendix, display the total egg counts from the inside and the outside of each heap. They show, that there is no obvious correlation between the location within the heap and the number of helminth eggs. In some cases there are more eggs in the inside sample, and in some cases the egg counts from outside are higher.

This hypothesis was tested applying a Wilcoxon Matched-Pairs Signed-Ranks Test to all sample pairs (inside and outside for each turning) in each heap. The results of the test are displayed in Table 3.4: for all heaps p is bigger than α (= 5%) i.e. H_0 cannot be disapproved. This means that the statistical test cannot detect a significant difference between the amount of helminth eggs inside and outside of the heaps. Therefore, it can be assumed that the two data sets (inside and outside) come from the same sample that was analyzed twice. Thus, the further investigation of the data uses the average of the values found for the inside and the outside of the heaps.

Table 3.4 Results of the Wilcoxon Matched-Pairs Signed-Ranks Test comparing the total numbers of helminth eggs inside and outside of the heap for each turning. n=number of pairs, W+ = sum of all positive ranks, W- = sum of all negative ranks, p= level of significance

Sample	n =	W+ =	W- =	p =
Cycle 5, turned according to temperature	11	41	25	0.505
Cycle 5, turned every 10 days	8	24	12	0.441
Cycle 6, turned according to temperature	15	30	90	0.094
Cycle 6, turned every 10 days	7	21.5	6.5	0.238

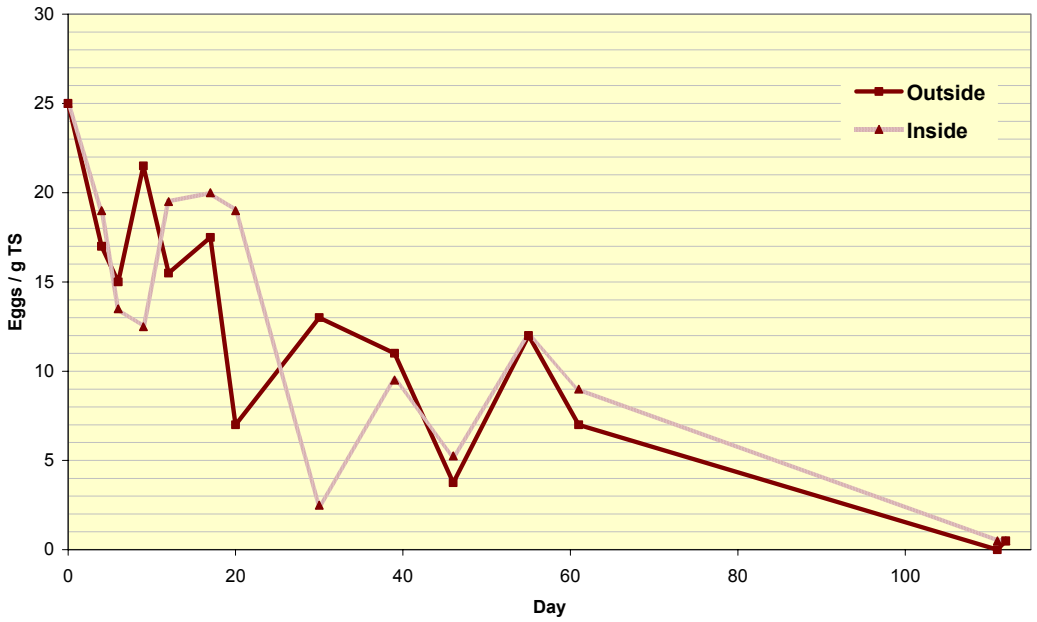


Figure 3.3 Cycle 5, Heap turned according to temperature: Comparison of the total number of helminth eggs (Ascaris and Trichuris) inside and outside of the composting heap

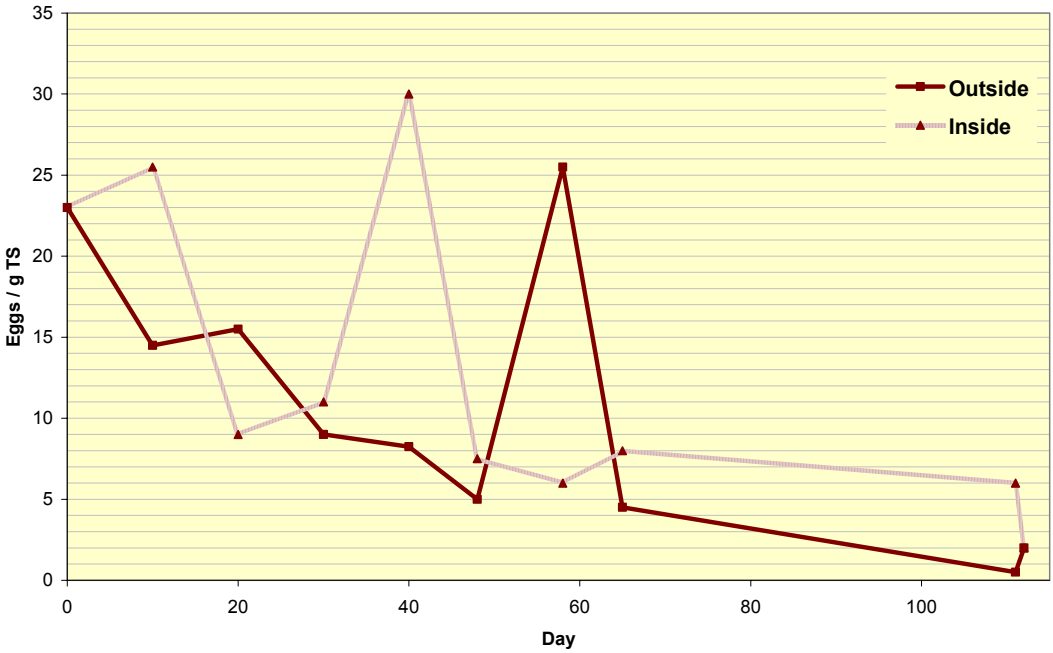


Figure 3.4 Cycle 5, Heap turned every 10 days: Comparison of the total number of helminth eggs (Ascaris and Trichuris) inside and outside of the composting heap

3.2.3 Reduction of the Different Helminth Egg Species

In all compost samples only Ascaris and Trichuris eggs were found, whereas Schistosomia eggs were always absent. Similar as in the sludge samples, also in the compost the number of Ascaris usually exceeds the number of Trichuris eggs; however, in Cycle 6 this difference is much smaller (see Figure 3.5 and Figure 3.6).

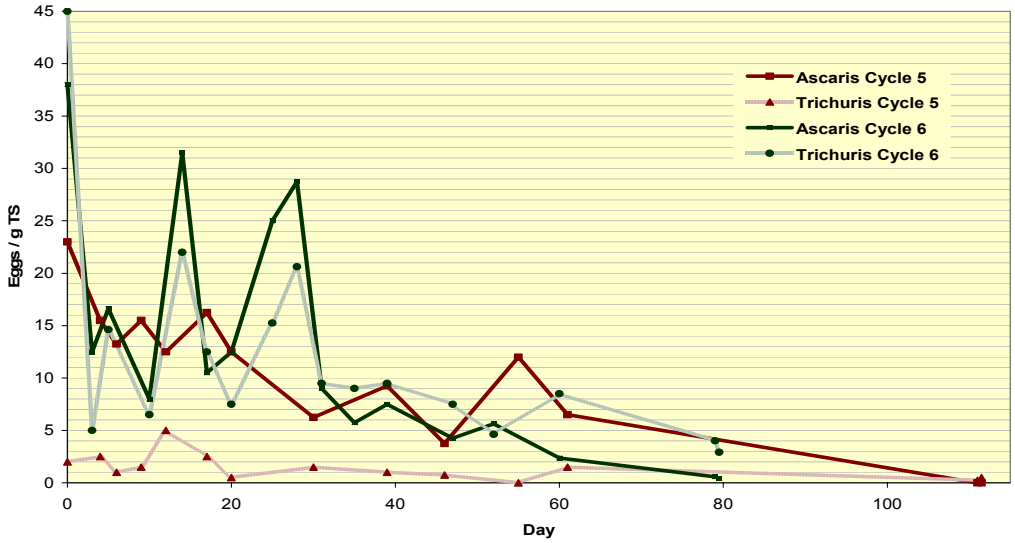


Figure 3.5 Comparison of the amount of Ascaris and Trichuris eggs in the heaps turned according to temperature (Cycle 5 & 6)

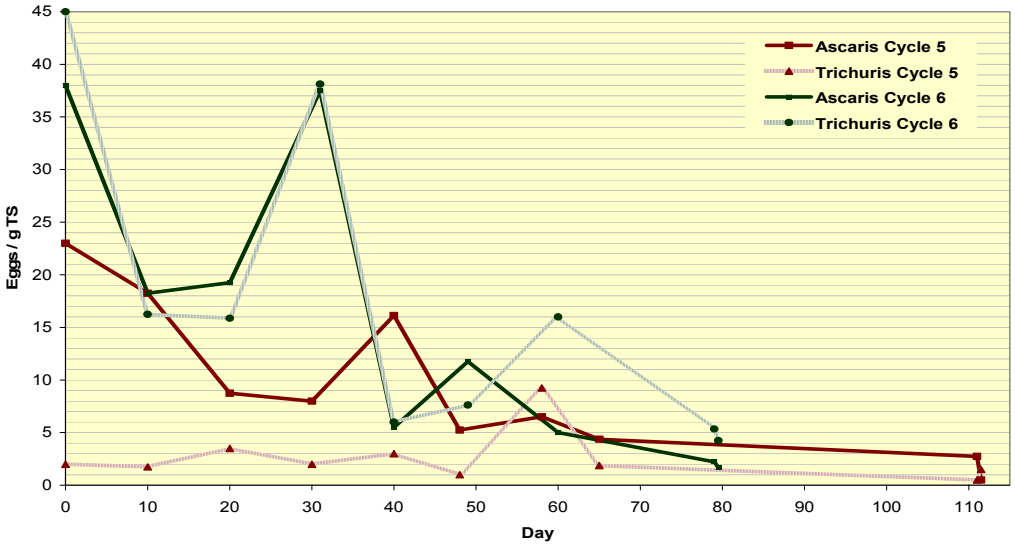


Figure 3.6 Comparison of the amount of Ascaris and Trichuris eggs in the heaps turned every 10 days (Cycle 5 & 6)

Figure 3.5, Figure 3.6, and Table 3.5 show that all species of helminth eggs are drastically reduced during the composting process. Only the reduction of Trichuris in Cycle 5 is not so great, but this could be due to the fact that there were only very few Trichuris eggs (2 eggs / g TS) at the beginning. In general the reduction rate of Trichuris seems to be a bit less than for Ascaris.

Already at the beginning of the maturation phase (after 60 days) the number of eggs is much smaller than in the beginning of composting (reduction of 6% to 94%). However, during the maturation phase this number is again reduced by 20% to 100%.

The maximum amount of eggs found in the end product is 6 eggs per g TS. Therefore the compost is by WHO guidelines (egg concentration of ≤ 3-8 eggs/gTS, Xanthoulis and Strauss (1991)) hygienically safe and can be used for agricultural purposes without restrictions.

Table 3.5 The reduction of the different helminth egg species (Ascaris and Trichuris) during the composting process. Compared are the amounts of eggs in the starting material, in the compost before the start of the maturation phase, and in the final product.

Cycle	Heap	Species	Start [Eggs/gTS]	Beginning Maturation * [Eggs/gTS]	End-product** [Eggs/gTS]	Reduction until Maturation	Total Reduction
Cycle 5	Heap 1	Ascaris	23	6.5	0.0	72%	100%
		Trichuris	2	1.5	0.5	25%	75%
	Heap 2	Ascaris	23	4.4	0.5	81%	98%
		Trichuris	2	1.9	1.5	6%	25%
Cycle 6	Heap 1	Ascaris	38	2.4	0.4	94%	99%
		Trichuris	45	8.5	2.9	81%	94%
	Heap 2	Ascaris	38	5.0	1.7	87%	96%
		Trichuris	45	5.4	4.3	88%	91%

* During the maturation phase the compost is not turned anymore. It starts 60 days after the beginning of composting.
** After 60 days of composting and 3 weeks (Cycle 6) to 6 weeks (Cycle 5) of maturation.

3.2.4 Influence of the Turning Frequency on the Helminth Egg Inactivation

One of the main questions of the present experiments was, whether the turning frequency influences the quality of the compost. By just looking at the material (texture, homogeneity etc) it becomes clear, that the turning frequency does have an impact on the product. Observations show that the compost from the heaps that are only turned every 10 days is much more heterogeneous and contains more clumps, whereas the compost that was turned according to temperature has smaller grains and a smoother texture. However, it would be interesting to know, whether this difference is also reflected in the helminth egg content of the compost.

In Figure 3.7 the relative helminth egg decrease of both heaps in both cycles is displayed. For Cycle 5 the starting material contained a helminth egg concentration of 25 eggs per g TS, Cycle 6 started with a concentration of 83 eggs per g TS. In both cases the helminth egg concentration is greatly reduced: down to 3-6 eggs/gTS in Cycle 6 and down to 1-2 eggs/gTS in Cycle 5. In Figure 3.7 no correlation between the die-off curves of the heaps that were turned with the same frequency

can be detected. Rather there seems to be a correlation between the die-off curves of the heaps coming from the same cycle.

However, the correlation is not obvious and this hypothesis will need further investigation. Therefore a Wilcoxon Matched-Pairs test was preformed to detect whether the egg die-off pattern in the individual heaps differs at all. The relative egg die-off in each heap was individually compared to the die-off in all other heaps (see Table 3.6). The test finds significant differences between Heap 1 Cycle 6 (turned according to temperature) and both heaps in Cycle 5. If we go back to Figure 3.7, we can see that the significance results from the fact that in Heap1 Cycle 6 the eggs die-off faster than in the other heaps. All other pairs do not differ significantly. Thus, significant differences can only be found between heaps coming from different cycles, but not between heap within the same cycle being subject to different treatments. Therefore it can be assumed that the cycle (i.e. starting material, climatologic parameters etc.) has a stronger influence on the course of the helminth egg die-off than the turning frequency.

Thus in conclusion it can be said that the present study cannot detect an influence of the turning frequency on the helminth egg die-off.

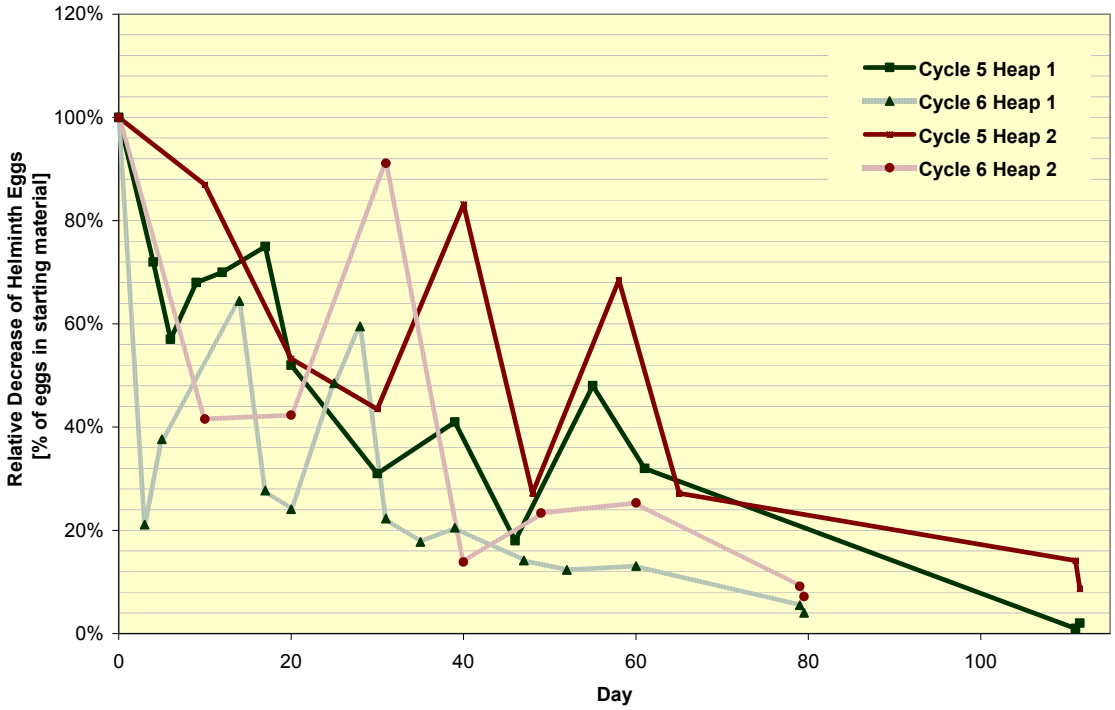


Figure 3.7 Comparison of the helminth egg die-off relative to the helminth egg contamination in the starting material in the different composting cycles (lines with same texture) and the different turning frequencies (lines with same color). The starting material in Cycle 5 had a helminth egg concentration of 25 eggs/gTS, Cycle 6 started with 83 eggs/g TS

Table 3.6 Results of the Wilcoxon Matched-Pairs test comparing the relative helminth egg die-off in the different composting heaps. Heap 1 of each cycle was turned according to temperature and Heap 2 was turned every 10 days. (N= Number of pairs, p= level of significance, significant differences are shaded)

	Cycle 5 Heap 1	Cycle 6 Heap 1	Cycle 5 Heap 2
Cycle 6 Heap 1	N = 8, **p <= 0.01562**	**----**	**----**
Cycle 5 Heap 2	N = 8, **p <= 0.6406**	N = 8, **p <= 0.007812**	**----**
Cycle 6 Heap 2	N = 8, **p <= 0.1953**	N = 8, **p <= 0.25**	N = 8, **p <= 0.25**

3.2.5 Correlation between the Heap Temperature and the Egg Die-Off

It is assumed that high temperatures are the main factor responsible for the die-off of helminth eggs (Feachem et al, 1983, p. 63). It would therefore be interesting to know, whether a correlation between temperature and helminth egg die-off really exists. However, such a comparison only makes sense for heaps where the die-off rates differ significantly, thus for the pairs Heap 1 Cycle 5 & Heap 1 Cycle 6, and Heap 2 Cycle 5 & Heap 1 Cycle 6 (see Table 3.6). In both cases the die-off rate for Cycle 6 Heap 1 is faster than for the heaps of Cycle 5. To relate this fact to the temperature it has to be shown, that the temperatures in these heaps also differ significantly. This is indeed the case: Table 3.7 shows that the differences in the temperatures are highly significant. The average temperature for Heap 1 Cycle 6 is 47.5°C compared to 43.2°C for Heap 1 Cycle 5 and 44.0°C for Heap 2 Cycle 5 (Table 3.7).

Thus, for Heap 1 Cycle 6 the temperatures are generally higher, and the helminth eggs die-off faster compared to the two heaps of Cycle 5 (see Figure 3.8). This would mean that higher temperatures lead to a faster helminth egg die-off. However, even though the temperatures in Heap 2 Cycle 6 are also significantly higher than the ones in both heaps of Cycle 5 (the significance is even bigger than for Heap 1, see Table 3.7), the helminth egg reduction in these heaps does not differ significantly (Table 3.6). From these results it can be concluded that the temperature inside the compost heaps could be one factor that influences the rate of the helminth egg die-off, but cannot explain the removal rates during the composting process.

One reason for this could be that the temperature differences in the heaps are too small to make significant difference for the helminth egg die-off (the overall average temperatures differ maximal 6.3°C, see Table 3.7). Also, in chapter “3.2.1 Compost Temperatures” it was shown, that all heaps are long enough above the theoretically necessary critical temperature for helminth egg die-off. If all helminth eggs are killed during this “critical” period, a significant difference between the helminth egg removal rates in the different heaps would not be expected.

Table 3.7 **Results of the Wilcoxon Matched-Pairs test comparing the average compost heap temperatures for each day. The overall average temperature (mean over 60 days of composting) is given in parenthesis. Heap 1 of each cycle was turned according to temperature and Heap 2 was turned every 10 days. (N= Number of pairs, W+= sum of all positive ranks, W-= sum of all negative ranks, p= level of significance)**

	Cycle 5 Heap 1 (Average T= 43.5°C)	Cycle 5 Heap 2 (Average T= 44.0°C)
Cycle 6 Heap 1 (Average T= 47.5°C)	W+ = 1042; W- = 183, N = 49 **p <= 1.982549e-05**	W+ =1050.5; W- = 174.5, N = 49 **p <= 1.176884e-05**
Cycle 6 Heap 2 (Average T= 49.8°C)	W+ =1270; W- =5, N=50 **p <= 1.06475e-09**	W+ = 1273.5; W- = 5.5, N=50 **p <= 8.549329e-10**

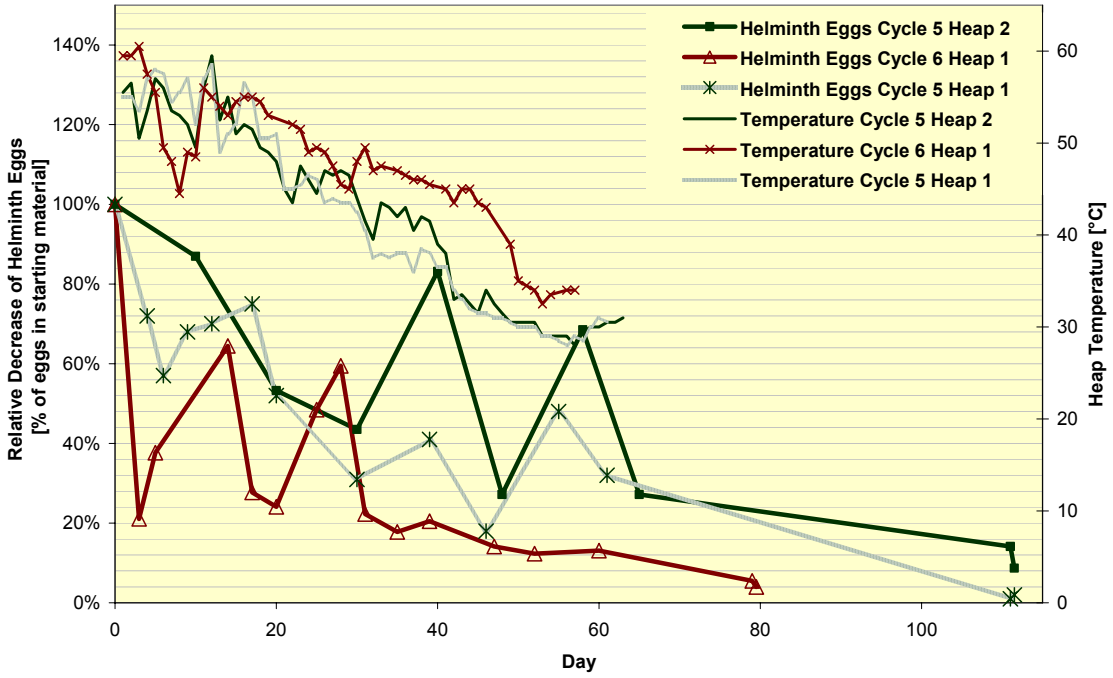


Figure 3.8 **Comparison of the helminth egg die-off relative to the contamination of the starting material and the average compost heap temperature. In Heap 1 of Cycle 6 (red lines) the overall temperature is higher and the helminth egg die-off is faster than in both heaps of Cycle 5 (green lines)**

3.2.6 Viability

The problem with all the data presented so far is, that it is not sure whether the counted helminth eggs are still viable (and thus infectious) or not. It could very well be that the eggs die off very early during the composting process, but that they are not decomposed for a long time. It would therefore be of interest to know, whether the counted eggs are viable or not. For Cycle 6 the viability of the Ascaris eggs was determined. Figure 3.9 displays the relative amount of viable Ascaris eggs (compared to the total Ascaris egg content) of the composting heaps. It can be seen, that the viability of the Ascaris eggs decreases in the course of composting. While it is around 60% in the beginning of the process it goes down to 0% at the end. Thus, not only the total amount of helminth eggs (Figure 3.5 and Figure 3.6) decreases, but also the viability of the eggs that are still there.

For the two composting heaps analyzed here, after 40 days, the viability is below 30% and after 50 days it is below 20%. However, these numbers might vary depending on the conditions and should therefore not be used as a general guideline.

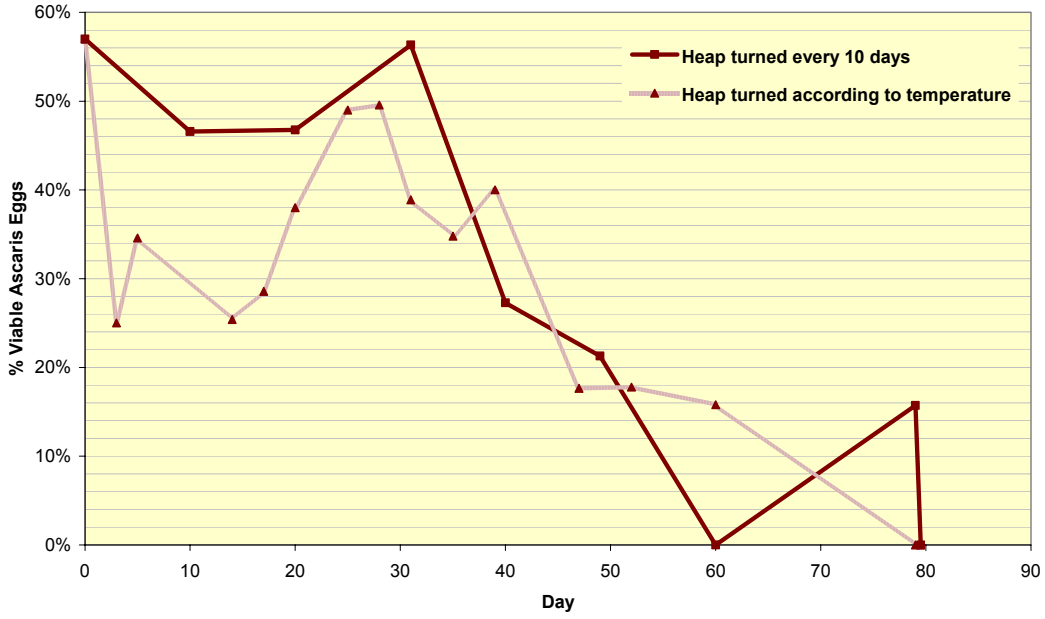


Figure 3.9 The relative amount of viable Ascaris eggs (compared to the total number of Ascaris eggs) in the two heaps of composting Cycle 6.

Because the detection of the viability was not without problems, 4 samples were analyzed with the Safranine as well as with the incubation method (see chapter 2.4.3). Table 3.8 shows the results of the comparison of the two methods. For 3 samples the results are quite similar (difference <= 6%), but for one sample the dying method results in a viability rate that is almost twice as high, as the one determined with the incubation method. In 3 out of 4 cases the dyeing method results in higher viability rates than the incubation method, but this does not necessarily mean, that the results

accomplished with the dyeing method are always too high. Generally these results do not give evidence that there is a big difference in the two methods and it is hard to say which method is more reliable.

Table 3.8 Comparison of the two methods (incubation for 3 weeks, dying with Safranine O) used to determine the viability of Ascaris eggs. For each sample the relative amount of viable Ascaris eggs was determined with both methods.

Sample	relative amount of viable Ascaris eggs as determined by	
	the incubation method	the dyeing method
T8 O	30%	56%
T9 O	44%	40%
T11 I	43%	44%
PTS 4	41%	47%

3.3 Composting Settled Sludge Mixed with Sawdust

3.3.1 Temperatures

To analyze whether helminth eggs could theoretically die-off in sludge from the sedimentation pond in Teshie mixed with fresh saw dust, the temperature inside the heaps was measured about every two weeks. Unfortunately, due to technical problems temperature monitoring only started three weeks after beginning of composting. Thus the first phase of composting, where temperatures would be highest was not monitored. The values observed afterwards are plotted in Figure 3.10. It is quite striking how low the temperatures are. They are never above 45°C, which means, that in theory (and if the temperatures were not higher in the non-monitored phase) the helminth eggs would not die-off (c.f. Figure 1.2). But not only the temperature maximum is very low, also the temperature minimum is much smaller than expected. The values go down to 23 °C which is below the average outside temperature for this period (November, average air temperature in Accra = 27.5°C, Müller, 1983).

Therefore it could be that there was a problem in the measurement of the compost temperature, and the data will not be further analyzed.

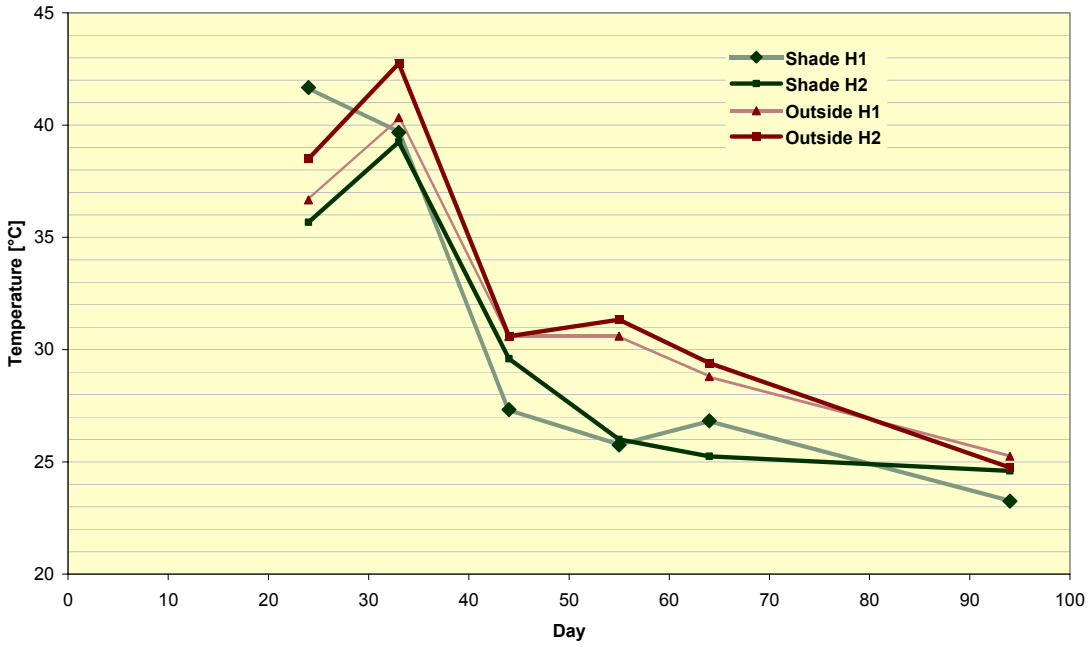


Figure 3.10 Temperatures observed inside the compost heaps formed out of settled sludge and saw dust. Composting started at day 1.

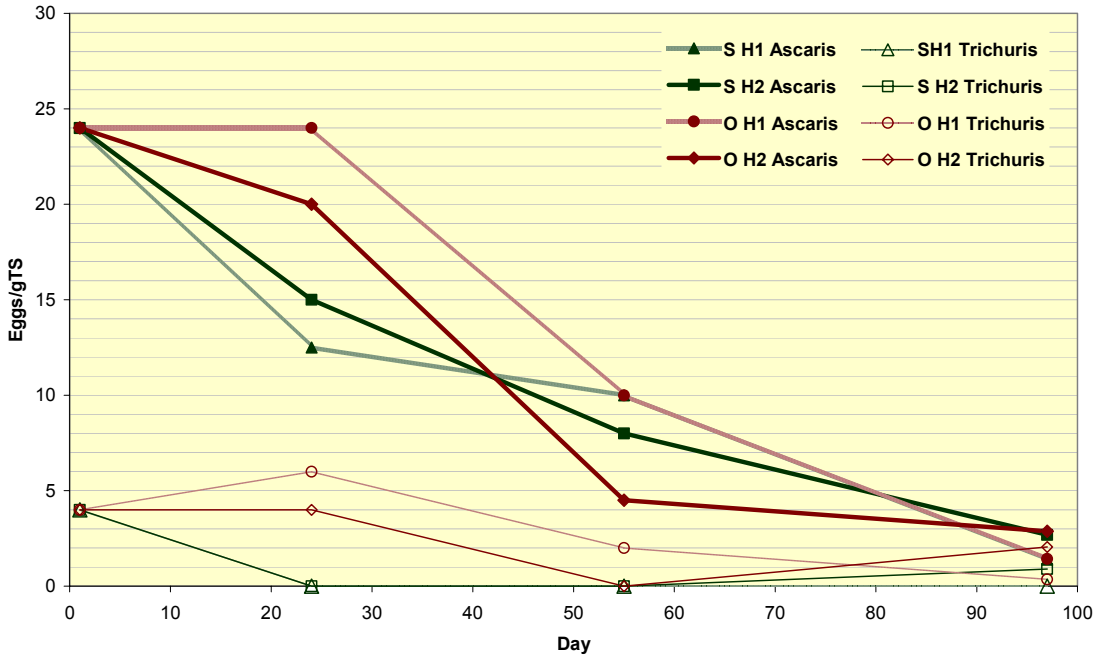


Figure 3.11 Decrease in Ascaris (bold lines) and Trichuris (thin lines) eggs in compost heaps formed out of settled sludge and saw dust. Two of the heaps were located under a shade (S, green lines) and two heaps were unsheltered (O, red lines).

3.3.2 Helminth Egg Species and Die-Off

The initial helminth egg concentration in the sludge from the settling tank is 28 eggs per gram total solids. This concentration is exceeds the WHO guidelines for materials used in agriculture by a factor 4 to 9. Therefore the sludge cannot be used directly, but will have to be treated to be sanitized.

In the starting material (sludge from the settling pond) and in the compost only Ascaris and Trichuris eggs were found. Schistosomia eggs were never present. As in the material used for co-composting the number of Ascaris eggs is much higher than the number of Trichuris eggs.

All kinds of eggs helminth eggs are drastically reduced during the three-month composting period. The amount of Ascaris decreases from 24 to 3-4 eggs per g TS and the amount of Trichuris is reduced from 4 to 0-3 eggs per g TS. Of the 28 eggs per g total solids in the starting material there are never more than 5 eggs left at the end of the composting process (Figure 3.11). Therefore the material now satisfies the WHO guidelines and can be used without precautions for agricultural purposes. The composting process has successfully removed the pathogens.

3.3.3 Influence of the Location of the Heap (Shade vs. Outside)

Two of the composting heaps were formed under a shade, and the other two were formed outside. The aim of this chapter is to see whether this location plays a role in the helminth egg die-off. Therefore the total amount of helminth eggs (Ascaris and Trichuris) in the heaps was compared. Because of the lack of data (only three measurements per heap) it does not make sense to perform a statistical test. If we look at Figure 3.11 there is no obvious difference between the heaps outside and the ones that were shaded. Sometimes more eggs can be found in the shaded heaps, but the next month there are more eggs in the outside heaps.

Thus, from the present data it is impossible to detect a difference in the helminth egg die-off rate in the heaps under the shade or outside. However, because climatic changes (rain, strong solar radiation etc.) could greatly influence these results, it would be important to restart another experimental series at the different time of the year.

3.3.4 Viability

For some compost samples, (but not the starting material) the viability of the Ascaris eggs was determined. The relative amount of viable Ascaris is displayed in Figure 3.12. Even though there is only a limited amount of data, it can be seen, that the viability generally decreases in the process of composting (from 60%-70% after 1 month of composting to 0%-50% in the end). Therefore, not only the total number of Ascaris eggs decreases (Figure 3.11), but also the relative amount of viable eggs.

Interestingly, however, the decrease in the viability is not as drastic as for the Kumasi co-composting heaps in Cycle 6. Where as in the Kumasi experiments the viability is always below 20% already after 50 days, in Teshie a viability below 20% is only reached in one of the heaps (Heap 1, shaded), and only after 100 days. It is hard to say, why this is the case. Many factors could have influenced this difference: the treatment of the heaps (the heaps in Teshie were turned only every month, compared to at leas every 10 days in Kumasi), the different starting materials

(sawdust instead of market waste), or the climatic conditions (in Teshie the temperatures are usually a bit higher than in Kumasi). However, there are not enough data to prove either of this assumptions and the difference the in viability decrease between the Kumasi and the Teshie experiments could also be purely coincidental.

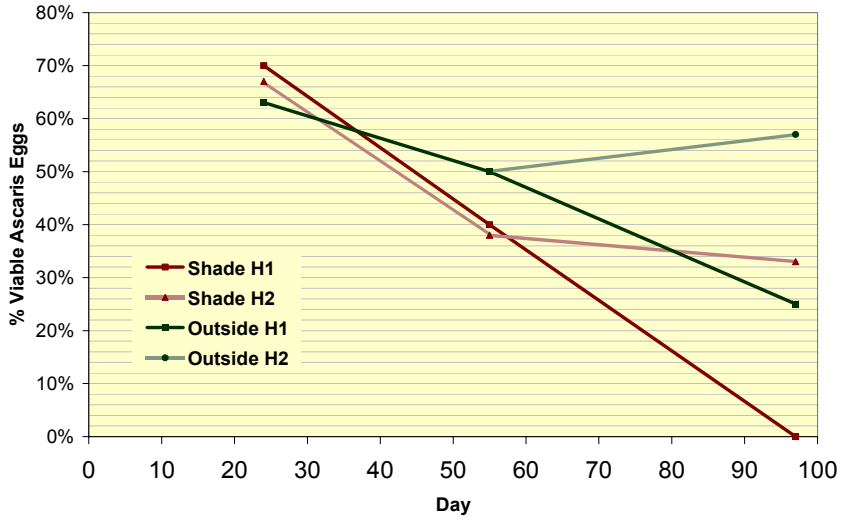


Figure 3.12 The relative amount of viable Ascaris eggs (compared to the total number of Ascaris eggs) in compost made out of settled sludge mixed with sawdust. The viability in the starting material was not determined; observations started only after 24 days of composting.

4 Discussion

4.1 Data Fluctuation

One of the most striking features of the data obtained in this study is the big fluctuation in the number of helminth eggs detected in the course of time. Because helminth eggs cannot multiply outside of the hosts body (Schwartzbrod, 2003), this has to be due to an analytical problem. One explanation that immediately comes to mind is the heterogeneity of the samples. Especially in the beginning of the composting process, the material was still very clumpy and the market waste was not really mixed with the fecal sludge. Therefore depending on the material analyzed the helminth egg content could be very high (if the sample contained only faecal sludge) or close to zero (if the sample consisted only of market waste). If this were the main reason for the strong fluctuation in the helminth egg number, then the fluctuation should decrease as the samples get more homogenous in the course of composting. This is, however, not always the case. It seems true for Cycle 6 Heap 1 (Figure 7.1) and to a lesser extent Cycle 6 Heap 2 (Figure 7.2), but for the two heaps in Cycle 5 the amount of helminth eggs still fluctuates at the end of the active composting phase (Figure 3.3, Figure 3.4).

On the other hand, the fluctuation in the amount of helminth eggs found in different samples could also be due to egg losses or equipment contamination during the sample preparation. Due to the results of the blank control experiments (see chapter 2.4.4) equipment contamination can be more or less excluded as a factor falsifying the results. However a loss of eggs during the preparation is likely to occur. For example Bean and Brabants (2001) found, that in only 4.5% of Ascaris eggs could be recovered from artificially contaminated composted bio solids. It can, however be assumed, that the recovery rate is not always the same, which would lead to a strong fluctuation in the results. The results from the “double count” controls (see Table 2.1) give an indication, that this could actually be the case. Results for samples counted or prepared twice can differ to al large extent. However, this does not sufficiently explain the data fluctuation, because if it were merely due to the loss of eggs in the sample preparation, then the samples that were only counted (but not purified) twice should give the same results. This is not the case, which could be due to a fact pointed out by Bowmana et al. (2003): In obtaining a sample of a liquid containing Ascaris eggs by pipette, extra caution must be taken to make sure it is a representative sample. Since the eggs are much denser than the aqueous solution they are in, they settle rapidly after the solution is mixed to randomize them. If the sample is taken at the top or the bottom of the container, the number of eggs in the sample will not represent a ‘‘true’’ random sample. Even though extra caution was paid to this fact during the loading of the Sedgwick rafter, it is still possible that it has influenced the results.

In conclusion it has to be said, that there is always an uncertainty in the number of eggs counted in a sample. It can, however be assumed, that the egg counts obtained are rather too low than to high. In order to get more reliable data, every sample should be analyzed several times. Because this is really time (and money) consuming it would be advisable to reduce the amount of samples e.g. analyze only one sample per month. This could be sufficient because the decrease of helminth eggs does not follow as step function but seems to be a continuous process (see also chapter 4.2).

4.2 Predictions for the Helminth Egg Die-Off

In order to make predictions on the process of the helminth egg die-off, it is necessary to find a function describing the actually observed data. It would be expected that the helminth egg die-off follows an exponential decrease because the process can be considered to be random (i.e. the probability of an eggs death, is independent of the death of other eggs. The same model is used for the nuclear disintegration).

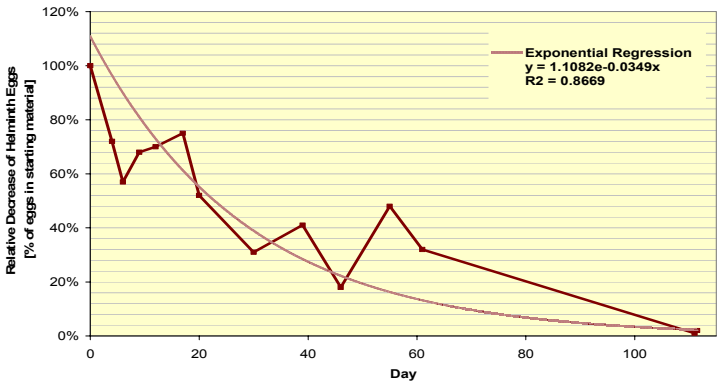
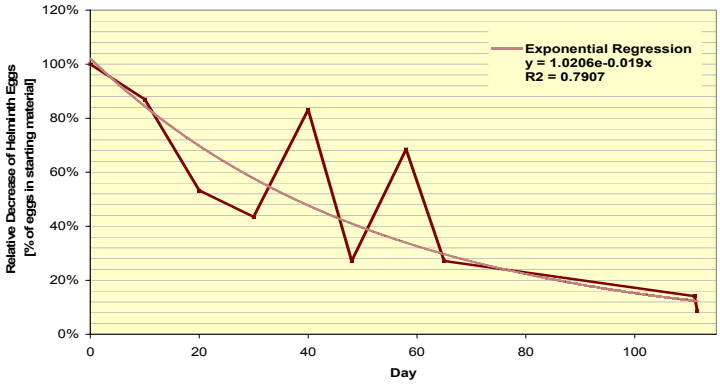
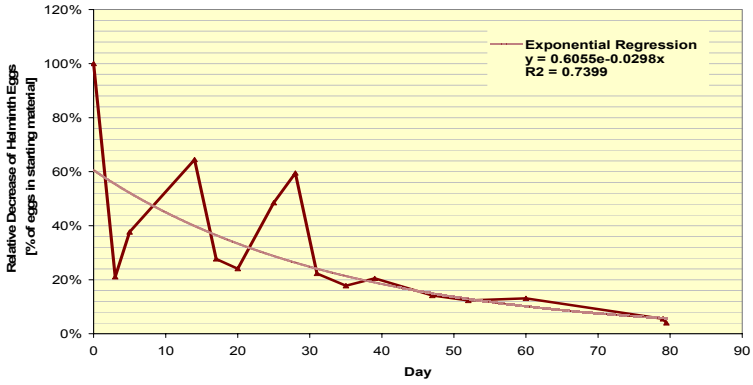
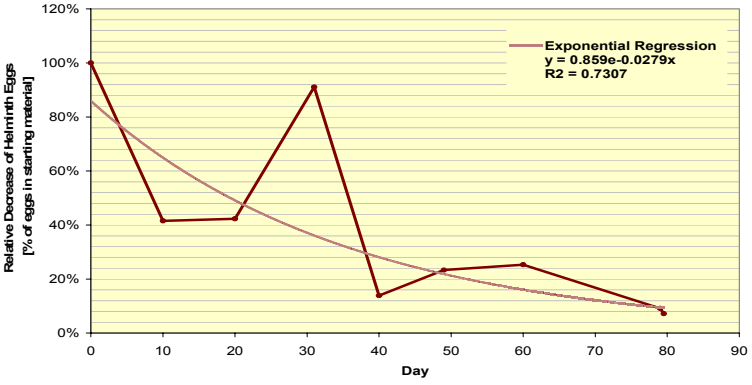
To test whether this assumption could be true the method of the least squares was used to find the function that best describes the decrease of helminth in the analyzed compost heaps. The curves were fitted to the data describing the die-off relative to the starting concentration, because this would allow comparing the different heaps. It turned out that, as expected, exponential functions best describe the course of helminth egg die-off in the compost. Values for R^2 laying between 0.7307 and 0.8669 (see Figure 4.1).

How can these functions help to predict the course of the helminth egg die off in a compost heap? The most important characteristic of the curve is its slope i.e. the term $e^{-a * x}$, because it determines the sped of the helminth egg die-off. A large coefficient (a) indicates a fast decrease in helminth eggs and a small coefficient stands for a slow decrease. This means that the die-off rate in Cycle 5 Heap 2 is the slowest (a=0.019) and in Cycle 5 Heap 1 it is the fastest (a=0.0349).

With these values it is possible to predict how long it will take until the helminth egg concentration in the compost is below 8 eggs/g TS and can thus without health risks be used in agriculture. Because the die off curves work with a relative decrease this depends on the helminth egg concentration in the starting material. In the dried sludge analyzed here, the helminth egg concentration ranges from 22 to 83 eggs per g TS. It can thus be assumed that usually the helminth egg concentration lies below 100 eggs/gTS.

Thus in the worst case, the initial egg concentration would be 100 eggs/gTS and die-off rate would be the slowest ever fond (a=0.019). In this case it would take 132 days until the compost would be safe to use. In the best case, however, the initial concentration would only be the lowest ever found (22 eggs /gTS) combined with the fastest egg die-off rate (a=0.0349). Then the compost would be hygienized already after 29 days. This means, that depending on the conditions and the starting material it will take one to four and a half month for the compost to be ready for use.

Figure 4.1 The curves obtained by the method of least squares best representing the relative helminth egg die-off in the compost heaps analyzed in this study

Cycle 5 Heap 1 $y = 1.1082e^{-0.0349x}$ $R^2 = 0.8669$	
Cycle 5 Heap 2 $y = 1.0206e^{-0.019x}$ $R^2 = 0.7907$	
Cycle 6 Heap 1 $y = 0.6055e^{-0.0298x}$ $R^2 = 0.7399$	
Cycle 6 Heap 2 $y = 0.859e^{-0.0279x}$ $R^2 = 0.7307$	

4.3 Viability

As described in chapters 2.4.3 and 3.2.6, it was not easy to determine the viability of the Ascaris eggs, because both methods that were used are subject to an uncertainty. It is therefore necessary to compare the results obtained in the present experiments with data found in literature. Table 4.1 gives an overview over helminth egg viabilities observed by different authors in different countries and in different kinds of starting materials.

Table 4.1 Literature review on the viability of helminth eggs in different kind of treatment plant sludges and biosolids.

Type of Sample	Location	HE Contamination (eggs/l):	Viability	Source
Biosolids treated in an anaerobic reactor	two differnet treatment stations in Curitiba, Parana State, Brazil	A: 611, T: 16 [1]	A: 44%, T: 20% [1]	Paulino et al. (2001)
		A: 295.6, T: 1.9	A: 38.7%, T: 3.6%	
Liquid portion of sludges treated in an anaerobic reactor	three differnet treatment stations in Curitiba, Parana State, Brazil	A: 57.8, T:0.3	A: 58.8%, T: 50%	Paulino et al. (2001)
		A:19.1, T: 1.1	A: 59%, T: 8.3%	
		A: 0.7, T: 0.1	A: 50%, T: 100%	
Sludge from a demonstration-scale combined UASB reactor - maturation pond system	Itabira city, Brazil		88% (in 1 year old sludge)	von Sperling et al. (2003)
Sludge samples from different locations in the sludge layer of a municipal wastewater stabilization pond	Mexicaltzingo, Mexico (South of Toluca in Mexico State).	74 (eggs/gTS)[2]	50% [2]	Nelson and Darby (2001)
		64 (eggs/gTS)	39%	
		48 (eggs/gTS)	28%	
		126 (eggs/gTS)	17%	
		141 (eggs/gTS)	19%	
		66 (eggs/gTS)	21%	
		98 (eggs/gTS)	14%	
		136 (eggs/gTS)	11%	
		87 (eggs/gTS)	4%	
		123 (eggs/gTS)	5%	
		118 (eggs/gTS)	1%	
		94 (eggs/gTS)	1%	
		80 (eggs/gTS)	0%	
Wastewater from an experimental WSP scheme	Extrabes, Campina Grande (Brazil)	1,000 eggs/l		Stott *et al*. (1994)
Biosolids from an experimental WSP scheme		1,400- 40,000 eggs/ gTS	2-8% after several years of storage	

Wastewater from WSP schemes	Chiclayo (Peru)	10-40 eggs/l		Klingel (2001)
Sludge from a primary facultative pond		60-260 eggs/gTS	1-5% after 4-5 years	
Septage	Asian Institute of Technology (Bangkok)	600-6,000 eggs/l		Koottatep, Surinkul (2001); Schwartzbrod (2000)
Biosolids form a pilot constructed wetland plant		170 eggs/gTS	0.2-3.1% after 3.5 years	
Sludge from drying beds in sewage treatment plant	Michalovce in the East Slovak Lowland		80.4% (fresh) 19.8% (after 3 month) 5% (after 8 month)	Plachy and Juris (1995)
	Poprad, Slovakia		36% (after 11 month)	

[1] A= Ascaris, T= Trichuris

[2] Ascaris eggs: Numbers given here are the average between the results obtained by the two different methods described in the paper

Table 4.1 shows that the viability of Ascaris eggs in sludge found in sewage treatment plants varies a lot. It ranges from 0% to 88%, where as the sludges with very low viability are probable quite old (sludge from sludge layer of stabilization pond, or stored sludge).

The present study found 40% to 82% of viable Ascaris eggs in fresh sludge (see Table 3.3). This amount is comparable with the data found in literature. The results obtained with the used viability test are thus plausible. On the other hand it can be said that with respect to helminth egg viability the sludge found in Kumasi has similar properties, as sludges found in different areas of the world (namely, South East Asia, Central and South America and Easter Europe). Therefore the results obtained in Ghana could be of interest for other countries as well.

4.3.1 Viability Reduction during Sludge Treatment

During the composting process of Cycle 6 the initial Ascaris egg viability of 60% drops to 30% after 45 days, to 20% after 50 days and to 0% within 80 days. This corresponds to a viability reduction of 50% after 45 days, 66% after 50 days and 100% after 80 days. How does this rate compare to sludges that are not treated but just stored? Table 4.2 gives an overview over helminth egg viability reduction rates for different storage conditions found in literature. The overview shows that at least 1 year of storage is necessary to kill off all the helminth eggs (zero viability), however, in some cases it takes even longer (up to 3 years). In the present experiments, all Ascaris eggs were dead already after 80 days. This means that co-composting is a fast and efficient method to reduce the amount of viable helminth eggs in sludges.

Table 4.2 Literature review on the reduction of the viability of helminth eggs in different kind of sludges and biosolids, stored for different periods

Type of Sample	Treatment	Reduction of the helminth egg viability	Source
Sludge artificially infected with *Toxocara canis, Trichuris vulpis, Trichuris suis, Ascaris suum* and *Hymenolepis diminuta*	Storage for 3 month at 25°C	5% -40%	O Donnel et al (1984)
	Storage for 1 year at 25°C	70%-100%	
Ascaris eggs in sludge	7 months of storage at 0 to 20°C	10%	Schatzle (1969)
Ascaris eggs in sludge	stored for 6 months at 4 to 20°C	0%	Stern and Farrell (1977)
Ascaris eggs in sludge	storage for 1 year	50%	Veerannan (1977)
	storage for 3 years	100%	
manure from dairy cows	stored in a pit for 56 days	40%	Plym-Forshell (1995)

4.4 Recommendations and Conclusions

The present study shows that fecal sludge coming from public toilets, septic tanks or sludge treatment plants in Ghana is highly contaminated with helminth eggs. However, co-composting this sludge with marked waste is a good tool to convert fecal sludge into a hygienically safe product that can be used as a fertilizer in agriculture. Also, the co-composting of settled sludge and saw dust is a possibility to inactivate the pathogens in sludge.

4.4.1 Temperatures

The temperatures reached inside the composting heaps were in all cases sufficient to kill the helminth eggs. It is thus sufficient to reach an average heap temperature of 43.5°C over the whole composting period sanitize the compost. However, temperatures of up to 58°C were reached, and it is hard to say whether the average or the maximum temperature is responsible for the die-off of the helminth eggs.

4.4.2 Turning Frequencies

The different turning frequencies did not significantly influence the process of helminth egg die-off. Only with respect to the sanitation of the product it would thus be enough to turn the heaps every 10 days. However, such a low turning frequency could negatively influence other characteristics of the compost (e.g. the texture).

4.4.3 Differences Between Inside and Outside

The rate of the helminth egg die-off is the same in all locations of the heap. It does not matter whether the sample comes from inside or the surface of the heap. This is probably due to the frequent turning of the heaps.

4.4.4 Time Needed to Reach a Save Product

Three month of composting were in all observed cases enough to reach a hygienically save product (i.e. less than 8 helminth eggs per g TS). With the data presented in this study it is possible to calculate the time theoretically in any given situation to sanitize the compost. In the worst case this would take 4.5 month and in the best case only 1 month is needed.

4.4.5 Viability of Ascaris Eggs

Not only the total amount but also the viability of Ascaris eggs decreases during the composting process. In average (over heaps of Cycle 6 in Kumasi, and all heaps in Teshie) the viability of the Ascaris eggs after 60 days is only 32% (SD = 20%) and after 90 days it goes to 19% (SD = 23%). Thus only a small portion of all counted helminth eggs are actually viable and infectious, which means that the product is probably save to use even faster than stated above.

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7 Appendix

7.1 Step-by-step Procedure for Helminth Egg Analyses

7.1.1 Reagents Needed for 1 Sample

For all preparations always use deionized H_2O.

1) 120ml $MgSO_4$, H_2O (specific density: 1.29 = 690g/l)

Preparation for 1 liter: take a bit less than 1l of H_20 and add 690g $MgSO_4$, stir well for about 30min (until everything is dissolved), heat if necessary with ca 50°C. Let cool down and fill up to exactly 1l. Measure the density.

2) 2l Phosphate-buffered H2O + OMO (The Nancy Method uses a detergent called Tveen20, because this canot be found in Ghana, it was replaced by the household detergent OMO)

Preparation for 1 liter: take 34g KH_2PO_4 per 1l of H_2O stir well and add ca 200mg of OMO (be careful of the bubbles)

3) 5-10 ml 0.1N H_2SO_4

Preparation for 1 liter: take a bit less than 1l H_2O and add 2.8 ml 95%H_2SO_4. Fill up to 1l with H_2O.

4) 14ml 0.1 N H_2SO_4 in ethyl alcohol (lipophilic/hydrophilic solution)

Preparation for 1 liter: take 350ml CH_3CH_2OH and add a bit less than 650 ml H_2O and 2.8 ml 95%H_2SO_4. Fill up to 1l with H_2O.

5) 6ml Ethyl acetate

6) 2 Drops of Safranine O (2.5% in H_2O)

7.1.2 Equipment

- Kitchen blender
- Centrifuge
- Water jet pump
- Microscope
- 80 mesh sieve
- 2l buckets
- 150 ml centrifugal tubes (alternatively Pyrex Tubes)
- 15 ml centrifugal tubes
- Pipettes
- Sedgwick-Rafter

7.1.3 Preparation

Determination of the total dry weight of the sample

Weight about 100g of sample (=Yg) in a dish (weight the dish first). Let the sample dry for 24h at 105°C. Let cool down and weight again, and subtract the weight of the dish (=Xg).

The percentage of dry matter is calculated as follows: TS = Xg/Yg

Separation of the eggs from the solid matter

➔ Weight a sample that corresponds to 4g of dry matter (i.e. 4g/TS). The amount of sample depends on the HE concentration in the sample. If the concentration of HE is very low you will have to start with 10g dry matter.
Alternatively you take 10l of wastewater, let settle for 5h and use the sediment for the following steps.

➔ Add 250ml of tap water to the sample and blend with a kitchen blender for 1 min at high speed.

Open particles to expose eggs to the treatment

➔ Pour the sample into a bucket (size at least 1l). Rinse the blender with phosphate buffer (reagent 2) and pour the rinse into the bucket. Fill the bucket up with buffer to nearly 1 liter.

➔ Let settle for 3h or over night (if a “cake” forms on top, stir slightly to destroy it)

Removal of big pieces of dirt (hair, toilet paper etc)

➔ Homogenize the sample and pour it through a sieve (80 mesh) and collect the percolate in a 2l bucket.

➔ Use a plastic pipe that is partly squeezed at the end to rinse the sieve with water, then rinse the sieve with phosphate buffer to make a total amount of 2l

➔ Let the sample settle for 3h or over night

Settling of the eggs

➔ Aspirate the supernatant using a water jet pump

➔ Mix the sediment by swirling and pour it into two 150ml centrifuge tube (alternatively Pyrex tube fitting into the centrifuge)

➔ Rinse the beaker 2 times with phosphate buffer (if there is too much material, rinse only once) and pour the rinsing into the centrifuge tube as well.

First centrifugation, eggs in sediment

➔ Centrifuge the tubes for 5min at 400G

➔ Pour off the supernatant, and keep the sediment

Flotation (egg will float because of the density of the solution)

➔ Add 60ml of $MgSO_4$ to the pellet in each of the tubes (thus 120ml to the whole sample) and re-suspend it by stirring carefully

➔ Wait for 10 minutes

➔ Centrifuge for 5min at 800G and let the centrifuge slow down (brake rate = 0)

➔ Keep the supernatant

Let the eggs settle in a diluted solution (density ca. 1)

➔ Pour the $MgSO_4$ supernatant in 1.5l of H_2O

➔ Let settle for 3h or over night

➔ Aspirate the supernatant using a water-pump

➔ Collect the sediment in a several 15ml centrifugal tubes

Second centrifugation

➔ Centrifuge the tubes for 5min at 800G

➔ Pour of the supernatant and collect the pellet in the centrifugal tube

➔ Re-group all the sediments in two 15ml centrifugal tubes and centrifuge again for 5min at 800G

Removal of lipophilic contamination

➔ Add 7ml of H_2SO_4-ethylalcohol (reagent 3) and 3ml of ethyl acetate (reagent 5) to the pellet in each centrifugal tube (i.e. 14ml of H_2SO_4-ethylalcohol and 6ml of ethyl acetate per sample)

➔ Invert several times

➔ Centrifuge for 5min at 660G

➔ Carefully remove upper layer (do not disturb the interface!!!!), then interface and then lower layer

Viability

➔ Add 1 drop of Safranine O to the pellet in each of the tubes. Shake.

➔ Wait for 5min

➔ Fill the tubes with H_2O

➔ Centrifuge for 5min at 800G

➔ Pour of the supernatant and re-suspend the pellet with H_2O. Centrifuge again at 800G

➔ Repeat until the supernatant is not red anymore

For storage and analyzes:

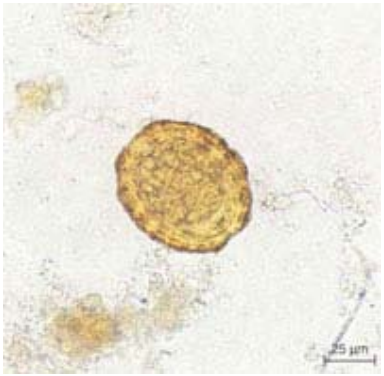
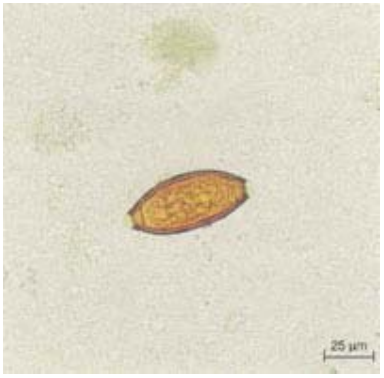
➔ Add H_2SO_4 to make 1ml (or until the solution is clear enough to be analyzed with the microscope)

➔ Use a Sedgwick-Rafter to count the eggs in 1ml of the sample

Helminth egg identification

➔ Use the following aid on the next page to identify the different helminth egg species.

Table 7.1 Key to the identification of Ascaris lumbricoides and Trichuris trichiura eggs. (After WHO 1994, and Schwartzbrod and Banas 2003)

	Ascaris lumbricoides	*Trichuris trichiura*
Size:	55-75μm * 35-50μm	50-55μm * 22-24μm
Shape:	Relatively polymorph, round to oval	Invariable. Oval with colorless bipolar prominences or plugs (like a lemon or an American football)
Color	Yellow to brown, due to the dirt sticking to the outer membrane, a decoated is completely colorless	Brown
Shell	Double membrane. Outer layer is thick and irregular (mamillated) and relatively fragile, can be missing (= decoated egg). Inner membrane is thick and transparent	Smooth with double membrane. Outer layer is brown, inner layer is transparent.
Interior	Granular mass, does not fill the egg completely. Can be embryonated i.e. a larva replaces the granular mass	Granular mass, fills the egg completely
Picture (Source: WHO 1996)		

7.2 Influence of the Location within the Heap

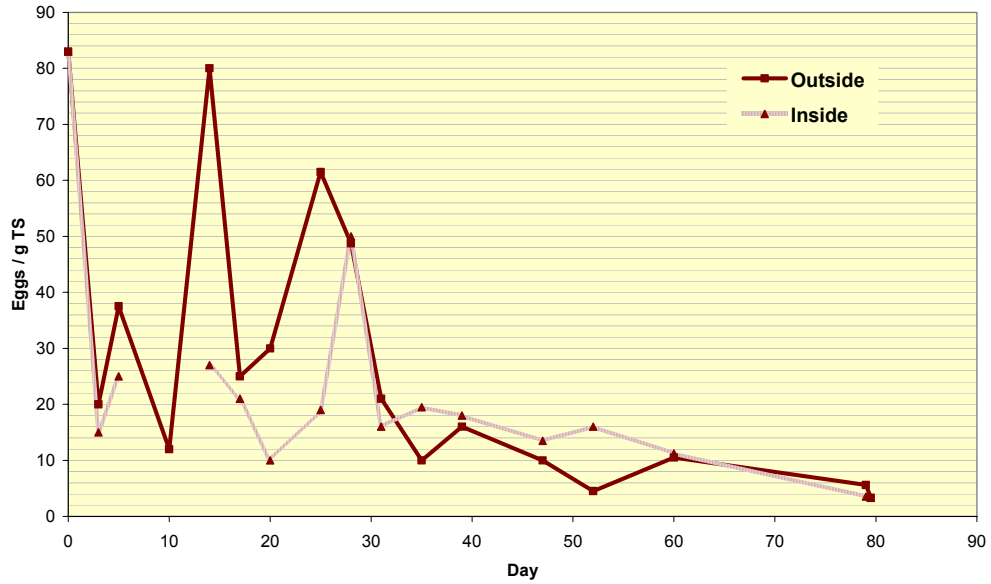


Figure 7.1 Cycle 6, Heap turned according to temperature: Comparison of the total number of helminth eggs (Ascaris and Trichuris) inside and outside of the composting heap Helminth Egg Species

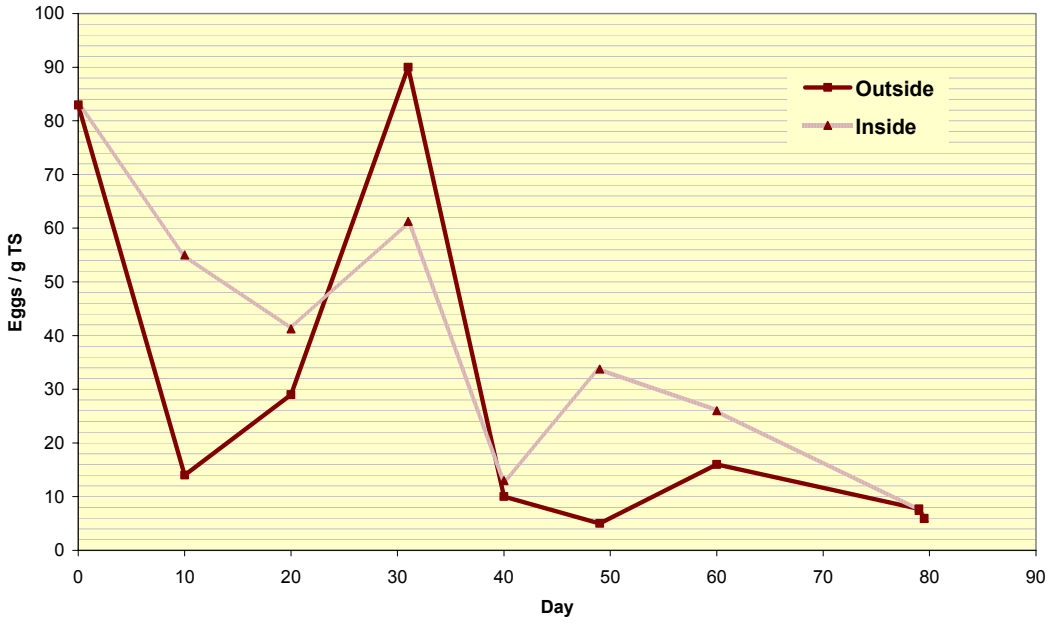


Figure 7.2 Cycle 6, Heap turned every 10 days: Comparison of the total number of helminth eggs (Ascaris and Trichuris) inside and outside of the composting heap Helminth Egg Species

7.3 Comparison between Helminth Egg Analyses in Nancy and Kumasi

Table 7.2 Comparison between Helminth Egg Analyses in Nancy and Kumasi (the same samples were analyzed in the two different laboratories)

		Nancy				Kumasi				Difference	
Sample*	Species	Total Eggs/g TS	Viable Eggs/g TS	% Viable Eggs	Total solids [%]	Total Eggs/g TS	Viable Eggs/g TS	% Viable Eggs	Total solids [%]	Total Eggs/g TS in Kumasi are X time higher	% Viable Eggs
PTS 1	Ascaris	133	50	38%		83	68	82%		0.62	44%
	Trichuris	24	3	13%		0				-	
	Capillaria	6	6	100%		n.d.**	n.d.				
	Toxocara	6	4	67%		n.d.	n.d.				
	Hymenolepis	3		0%		n.d.	n.d.				
	Total	**172**	**63**		**5.33%**	**83**	**68**		**5.62%**	**0.48**	
PTS 2	Trichuris	91	47	52%		n.d.	n.d.				
	Capillaria	30	27	90%		n.d.	n.d.				
	Taenia	7		0%		n.d.	n.d.				
	Total	**128**	**74**		**3.76%**				**4.54%**		
Septic 1	Ascaris	13	3	23%		30	15	50%		2.31	27%
	Trichuris	10	0	0%		5	n.d.			0.50	
	Toxocara	3	0	0%		n.d.	n.d.				
	Total	**26**	**3**		**2.51%**	**35**	**15**		**2.85%**	**1.35**	
Septic 2	Ascaris	94	49	53%		n.d.	n.d.				
	Trichuris	24	14	58%		n.d.	n.d.				
	Toxocara	1	0	0%		n.d.	n.d.				
	Total	**120**	**64**		**0.50%**	**0**					
FS Cycle 6	Ascaris	124	46	37%		150	72	48%		1.21	11%
PTS:Septic	Trichuris	65	16	25%		92	n.d.			1.42	
= 1 : 2	Toxocara	3	0	0%		n.d.	n.d.				
	Total	**192**	**62**		**1.40%**	**242**	**72**		**2.84%**	**1.26**	
FS Cycle 7	Ascaris	9	0	0%		32	24	75%		3.56	75%
PTS:Septic	Trichuris	6	0	0%		3	n.d.			0.50	
= 2 : 1	**Total**	**15**	**0**		**2.77%**	**35**	**24**		**2.59%**	**2.33**	
DS Cycle 7	Ascaris	31	0	0%		16	8	50%		0.52	50%
PTS:Septic	Trichuris	10	0	0%		6	n.d.			0.60	
= 2 : 1	Toxocara	7	0	0%		n.d.	n.d.				
	Total	**48**	**0**		**32.23%**	**22**	**8**		**28.4%**	**0.46**	

* PTS = Public Toilet Sludge, Septic = Septic Tank Sludge, FS = Fresh Sludge, DS = Dried Sludge
** n.d.= not determined