

# ADDIS ABABA UNIVERSITY SCHOOL OF GRADUATE STUDIES

## Assessment of Intestinal Parasites in the Effluent Slurry of Toilet-Linked Biogas Digesters

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## TABLE OF CONTENTS

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## TABLE OF CONTENTS

	Page
Acknowledgements	i
Content	ii
List of tables	iii
List of figures	iv
Abstract	v
	
1. Introduction	1
2. Literature Review	4
2.1. Intestinal parasites	4
2.2. Contamination of environment by intestinal parasites	4
2.3. Environmental impact of digesters	7
2.4. Anaerobic digestion of influent slurries in the digester	7
3. Objectives of the study	9
3.1. General objective	9
3.2. Specific objectives	9
4. Materials and Methods	10
4.1. Biogas digesters	10
4.2. Study areas and population	10
4.3. Sample collection	10
4.4. Measurements	11
4.4.1. <i>pH</i>	11
4.4.2. <i>Flow Rate</i>	11
4.4.3. <i>Temperature</i>	11
4.4.4. <i>Total solid</i>	11
4.4.5. <i>Free Ammonia</i>	12
4.5. Identification and enumeration of parasites	12
4.6. Statistical analyses	13
5. Results	14
5.1. Monitoring of digesters	14
5.2. Presence of eggs of intestinal parasites	20
5.2.1. <i>Asko</i>	21
5.2.2. <i>Lideta</i>	26
5.2.3. <i>Bisrate-Gebriel</i>	31
5.2.4. <i>Fitche</i>	36
5.2.5. <i>Awassa</i>	41
5.3. Protozoan parasites	45
6. Discussion	47
7. Conclusions and Recommendations	49
8. References	50

List of tables	Pages
Table 1. pH of influent and effluent slurries	14
Table 2. Retention days of influent slurries in digesters	15
Table 3. Temperature range of sites and digesters in degree Celsius	16
Table 4. Percentages of total solids of influent slurries	17
Table 5. Free ammonia concentration level of selected samples of influent and effluent slurries with respect to measured variables	18
Table 6. Correlation coefficient (r) between measured variables and elimination of <i>A. lumbricoides</i> , <i>T. trichiura</i> and <i>T. saginata</i> regardless of seasons and sites	19



List of figures

Pages

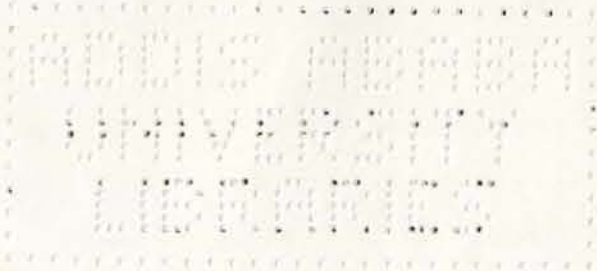
Figure 1 (a to d). Percentage of egg counts per ml in the influent and effluent slurries of **Asko** digester during the four seasons (*Kiremt, Tseday, Bega and Belg*) 21-24

Figure 2 (a to d). Percentage of egg counts per ml in the influent and effluent slurries of **Lideta** digester during the four seasons (*Kiremt, Tseday, Bega and Belg*) 26-29

Figure 3 (a to d). Percentage of egg counts per ml in the influent and effluent slurries of **Bisrate-Gebriel** digester during the four seasons (*Kiremt, Tseday, Bega and Belg*) 31-34

Figure 4 (a to d). Percentage of egg counts per ml in the influent and effluent slurries of **Fitche** digester during the four seasons (*Kiremt, Tseday, Bega and Belg*) 36-39

Figure 5 (a to d). Percentage of egg counts per ml in the influent and effluent slurries of **Awassa** digester during the four seasons (*Kiremt, Tseday, Bega and Belg*) 41-44



## ABSTRACT



Five toilet-linked biogas digesters working with in the range of ambient temperature were assessed to determine the presence of intestinal parasites in the effluent slurry considering the four seasons. These were in Addis Ababa (at Asko, Lideta and Bistrate-Gebriel), at Fitcha and Awassa. Retention days were determined through recording the flow rate of influent slurries. Eggs of intestinal parasites of the influent and effluent slurry from the digesters were identified by taking samples when the influent channeled into the digester at the mixing pit and effluent slurry away through the reservoir for each season. Eggs of *Ascaris lumbricoides*, *Trichuris trichiura* and *Taenia saginata* were commonly detected in the influent slurry of each site. Similarly, eggs of *Ascaris lumbricoides*, *Trichuris trichiura* and *Taenia saginata* were observed in effluent slurry of each site. The quantity of eggs in Kiremt was higher than Bega in the effluent slurry at all sites. Similarly, the quantity of eggs in the effluent slurry of Fitcha digester was higher than Awassa. These were due to significant positive correlation of temperature with reduction of eggs. In general, regardless of species and based on average removal per season, only less than half of the total observed eggs of the influent slurry were eliminated except Awassa digester in which case sixty percent were removed. Regarding protozoan cysts, *Entamoeba* species showed better elimination. However, *Cryptosporidium* species was found in the effluent slurry collected from Lideta, Awassa and Asko digesters during *Tseday* and *Belg* seasons. In conclusion, regardless of seasons a considerable quantity of eggs of *Ascaris lumbricoides*, *Taenia saginata* and *Trichuris trichiura* were observed in the effluent slurry of all the studied biogas digesters. Thus, there is greater risk of utilizing effluent slurry for gardening without any secondary treatment. Treatment options are indicated.

## 1. INTRODUCTION

Most of the problems of pollution may be regarded as the other aspect of human activity. Among these, the production of waste materials, some from food processing, some from the disposal of human excreta, but increasingly those from industrial production and from the products of industry (Ewer and Hall, 1978). Therefore, pollution of the environment is generally regarded as change brought about in the systems by various activities of humans. Effluent slurries from different sources that largely and actually discharged to the natural ecosystems bring undesirable organisms. The harmful effects of these organisms can be removed by allowing growth of microorganisms and can be discharged without further negative effects on the natural ecosystems. This biological treatment of potentially polluting wastes is mainly carried out by growth of microorganisms under controlled conditions. The microorganisms can grow and metabolize the wastes using oxygen or in the absence of oxygen, and it is this latter microbial metabolism and waste treatment that is called anaerobic digestion (Hobson and Wheatley, 1993).

According to Hall & Hobson (1988), anaerobic digestion is a microbiological process but it isn't a unique man made process, that is, the same reactions are carried out in nature, in soils and streams and in the oceans. The digester (plant) is simply a means by which this process can be concentrated, accelerated and its product (methane) is harvested and put to useful purposes (Mara, 1983).

The first application of the system was in the natural decay of human excreta in earth closets but having observed the over all process, men provided conditions in which the process could be more nearly optimized in the septic tank and its various types. From this came the more efficient anaerobic digester. In connection with this, the microorganisms carrying out the reactions in anaerobic digestion are bacteria that live without oxygen and may, indeed, be killed by oxygen. There is difference in tolerance to oxygen among anaerobic bacteria, but many of the digester bacteria are amongst the anaerobes, which are the least tolerant of oxygen (Pholand, 1971).

The feedstocks of anaerobic digesters vary in physical form as well as in chemical composition. They can be municipal wastes of animal excreta or plants, either residue from crops or crops grown for digestion and biogas production. All these may contain fibrous plant material of the

residues of plants that have been partially digested in human or animal digestive tracts, and the main primary substrate of the digester bacteria are these plant fibers (Hobson and Wheatly, 1993).

The main bacterial population and the reactions of the digester are determined by the nature of the feedstock. During the process, there are series of linked reactions providing energy and substrates for bacterial growth and leading to the final formation of methane and carbon dioxide. Here, the primary energy-producing reactions are breakdown of carbohydrates. If the feed is particulate animal tissues, the reactions will be similar but the balance will be moved to more fat and protein degradation. On the other hand, if the feed contains mainly dissolved sugars the primary hydrolytic reactions will not be needed and the reaction chain will start at the fermentation of simple sugars. However, in each case there is a degradation of substances that could be metabolized (Martin, 1991).

Wastes from different sources contain very large numbers of the bacteria in human and animal excreta. They are collected and fed to the digester. Digester feedstocks thus contain varying numbers of many species of bacteria, which may multiply in the digester. Fecal wastes contain at least small numbers of the bacteria needed to carry out the digestion (Hobson and Wheatley, 1993).

The biogas technology was introduced into Ethiopia as early as 1971, when the first batch type digester was constructed at Ambo Agricultural College. However, progress on the dissemination of biogas technology had been quite slow for various reasons. According to experts of Ethiopian Rural Energy Development and Promotion Center, so far around 500 digesters have been constructed in the country, which are mainly fixed dome and Indian types. However, fixed dome type is spreading as it has better cost of construction and durability than Indian type (Sasse, 1988). To this end, Non-Governmental Organizations, like Institute of Sustainable Development, Women and Children Development Organization and other similar organizations have already implemented the digester. In fact, governmental organizations, such as Environmental Protection Authority (EPA) are urging the above-mentioned bodies for their involvement in the expansion of the technology. However, there is no study conducted showing the effectiveness of biogas digesters to eliminate intestinal parasites in the effluent slurry in the country.

Assuming that the digester kills all pathogens (Li, 1987), the current practice is to utilize effluent slurry directly from digesters (from digesters of Asko, Awassa, Fitch and Lideta,) for cultivating vegetables and discharging to the stream (from digester of Bistrate-Gebriel) with out any further treatment. However, works in other countries showed the need for further treating effluent slurry of biogas digesters working with in the range of ambient digester temperature since it often contains ova and cysts intestinal parasites.

... of *Isospora* parasites (Chen et al., 1981) in animal wastes, especially those with widespread poultry and low standards of hygiene, the total number is considerably greater than that (Jirathakul et al., 1978). Approximately 1.2 billion people (25% of the world's population) are infected with *Isospora* (Crompton, 1999). This is associated to developing countries because these areas have poor sanitation and hygiene conditions where the eggs survive for long time (Gibson et al., 1977).

In the same manner, wide range of intestinal parasites infect the human intestinal tract. Most of these are worldwide in their distribution, but the range of species and their prevalence is higher in developing areas with low levels of sanitation and hygiene, as compared to industrial. The principal species affecting the human intestinal tract are *Giardia lamblia*, *Cryptosporidium parvum*, *Cyclospora cayentensis*, *Isospora belli* and *Cryptosporidium parvum*. Most of these intestinal parasites are oval or spherical intestinal parasites and their presence in a fecal specimen indicates that the person has recently ingested fecal material (Gibson and Gaido, 1993).

### 2.2 Contamination of environment by intestinal parasites

Fecal or sludge water associated with infectious organisms are common waterborne zoonotic agents worldwide have spread them. Some zoonotic disease require periods of external incubation before they become infective to the environment. *Coccidiosis*, *is* and *Isospora* feces and water are important vehicles for transmitting such zoonotic cysts and ova (Gibson et al., 1977).

Human beings can be infected from a variety of environmental sources, such as water, food and feed. In some cases, directly which means a greater number of fecal ova are available. A quantitative collection of numerous infections, zoonotic and zoonotic, has been done in the contamination of pasture water and feed with zoonotic parasites. The presence of zoonotic contamination

## 2. LITERATURE REVIEW

### 2.1. Intestinal parasites

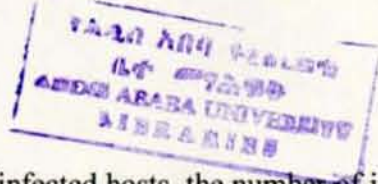
A wide range of intestinal parasites infect the human intestinal tract. At least half the world's population is infected with one or more species of helminthes parasites (Guerrant *et al.*, 2001). In tropical countries, especially, those with widespread poverty and low standards of hygiene, the rate of infection is considerably greater than this (Feachem *et al.*, 1978). Approximately 1.5 billion people (25% of the world's population) are infected with *Ascaris* (Crompton, 1999). This is concentrated in developing countries because these areas have poor sanitation and suitable environment where the eggs survive the longest time (de Silva *et al.*, 1997).

In the same manner, wide range of intestinal protozoa infects the human intestinal tract. Many of these are worldwide in their distribution, but the range of species and their prevalence is higher in developing areas with low levels of sanitation and hygiene, be temperate or tropical. The protozoan species infecting the human intestinal tract are *Giardia intestinalis*, *Isoospora belli*, *Cyclospora caytanensis*, *Entamoeba histolytica/dispar* and *Cryptosporidium* and microsporidia species. Most of these intestinal protozoa are strict or opportunistic intestinal parasites and their presence in a stool specimen indicates that the patient has ingested infected food or water (Mills and Goldsmid, 1995).

### 2.2. Contamination of environment by intestinal parasites

Food or drinking water contaminated with infective organisms can transmit infection when susceptible host ingest them. Some transmissible stages require periods of external maturation before they become infective in the environment. Contaminated soil, herbage, food and water are important vehicles for transmitting ova, larvae, cysts and oocysts (Feachem *et al.*, 1978).

Human beings can be infected from a variety of contaminated sources, such as water-borne and food-borne routes currently which create a greatest concern public health concern. Water-borne outbreaks of protozoan infection, effluent slurry spraying, etc, are due to the contamination of potable water and food pose significant problems. The potential for environmental contamination



depends upon a variety of factors including the number of infected hosts, the number of infective stages excreted, human activity, socio-economic and ethnic differences in behavior, geographic distribution, sanitation, safety of drinking water sources and supplies, climate and hydrology of the area (Smith, 1998).

Human activity and difference in lifestyles can influence the environmental matrices contaminated. For example, defecation by infected individuals on open field leads to the contamination of soil, fingers, hands, etc. (Feacham *et al.*, 1983). The use of untreated feces as a fertilizer, especially for vegetables, which receive minimal cooking prior to consumption, leads to contamination (Pawlowski, 1989). In the same manner, the use of effluent slurry, which is not properly treated, for irrigation leads to contamination of soil, food and water as does defecation by agricultural workers in or near the fields in which they work (Adams *et al.*, 1999).

Furthermore, parasites with both human and non-human reservoir hosts (for example, *Cryptosporidium* sp.) can increase environmental contamination following infection of hosts. Thus, the sources of contamination can be point sources, such as infected hosts, non-point sources such as effluent slurry spraying and run-off from contaminated land (Smith, 1997).

Regarding survival in the environment, helminthes ova are well tolerated to prolonged survival in the environment, with *Ascaris* ova being amongst the most resistant of intestinal pathogens. For instance, infective *Ascaris* ova can remain viable for more than one year, while those of *Trichuris* can remain viable for a year. However, cysts and oocysts survive less well in the environment than ova (Smith, 1998).

Digesters may have unwanted role of spreading intestinal parasites. This is due to the fact that pathogens in general and parasites in particular survive longer when the temperature of a digester is lower. Since higher temperature is accompanied by enhancement of free ammonia which is toxic and shortens the chance of survival of pathogen (DEP, 2000). To this end, as it is indicated by WHO (1989), the optimum temperature level for the digestion process with reference to the most resistant or indicator organism, i.e., *Ascaris lumbricoides*. In general, if there is a need to use the effluent slurry for land application, they recommend the digester should be at thermophilic level (Hays, 1977 cited in FAO, 1992). Thermophilic digestion at 53<sup>0</sup>C reduces

parasites to undetectable level, particularly parasite eggs (Bitton, 1999). However, under ambient temperature condition (10-29<sup>0</sup>C), several months are required before die off is assured (Ellard *et al.*, 1983); otherwise, mesophilic digestion temperature in general could be the source of environmental pollution because eggs such as *Ascaris lumbricoides* and *Trichuris trichiura* could survive well (Bitton, 1999).

### 2.3. Environmental impact of digesters

Although the primary objective of most biogas digesters is to produce methane, effluent slurry of digesters has additional advantage such as fertilizing the soil. The effluent slurry from a biogas plant is highly praised for its fertilizing value. As compared to traditional handling, the effluent slurry of the biogas plant contains more total nitrogen and more ammonia nitrogen (Van Buren, 1980).

Anaerobic digestion not only breaks down plant materials into biogas, it also releases plant nutrients, such as nitrogen, potassium, phosphorus and converts them into a form that can be easily absorbed by plants. However, while the value of biogas effluent slurry as a fertilizer is well known to biogas workers, most biogas programs have given it too little emphasis (Fulford, 1988). In line with this, in Ethiopia, gardening is being tried by the enforcement of Non Governmental Organizations. In addition, there is also a plan to use by some institutes such as prisons.

Now days, a major source of concern throughout the world is the safe treatment and disposal of wastes. Regarding biogas digesters, these are primarily concerned with treating organic wastes in general and excreta (human and animal) in particular, as they are potentially degradable. Meanwhile, hazards associated with treatment processes depend on the incidence of various organisms, survival rates in storage and discharge to the environment. In this regard, as mentioned above, that is, since there is a plan for utilization of effluent slurry and even discharge to water bodies, low temperature processes must be evaluated carefully (Pylo, 1976). In general, temperature is a key factor for complete fermentation and reduction of pathogens through the process (Barnett *et al.*, 1978).



## 2.4. Anaerobic digestion of influent slurry in digesters

Digestion of influent slurry takes place in a device called biogas digester that may consist of excreta or kitchen residue or similar materials (Mattocks, 1984). Of the many forms of biogas types, fixed dome has gained widespread acceptance by its significance (Van Buren, 1979). It is installed underground and works with the principle of displacement in order to keep continuous flow of influent slurry into and effluent slurry out of the digester (Fulford, 1988).

The digestion of the influent slurry, so as to give methane and effluent slurry is a complicated process that involves microorganisms (Hobson and Wheatley, 1993) and influenced by several factors. In this regard, bacteria need water and other suitable environmental factors. However, too much and too little water will affect the digestion process (FAO, 1978). Therefore, influent slurry with a solid content of 5-10% is particularly well suited for biogas plants (Sasse, 1988). According to Hobson and Wheatley (1993), apart from water bacteria require suitable conditions of pH to grow optimally. A successful pH range for the digestion is between 6.0-8.0 but efficient digestion occurs at a pH near neutrality (UNU, 1979). Operating temperature is another factor influencing digester efficiency. A digester can operate in three temperature ranges at which bacteria can grow. Generally speaking, different species of bacteria grow in the different temperature ranges. These ranges for growth are from 0<sup>0</sup>c to about 15<sup>0</sup>c, from 15<sup>0</sup>c to 45<sup>0</sup>c and above 45<sup>0</sup>c up to 65<sup>0</sup>c. These ranges are known as psychrophilic, mesophilic and thermophilic ranges, respectively. Bacteria need also carbohydrate and nitrogen for obtaining energy and synthesis of cell constituents through the metabolism of nitrogenous substances such as proteins, non-protein nitrogenous substances (Rivard *et al.*, 1988). Digester bacteria attack these substances to give ammonia as a final product (Jain *et al.*, 1988). Animal wastes, such as human excreta, are rich in nitrogen and provides nutrient for growth and multiplication of anaerobic microorganisms (Shuler, 1978).

In addition to the above-mentioned environmental factors, retention days influence the digestion process. Retention time (day) is the average number of days a unit volume of influent slurry is to remain in the digester, that is, it is the volume of material already in the digester divided by the average amount of incoming daily influent slurry (Mattocks, 1984 and Fulford, 1988). Therefore, imperfectly digested slurry may come out if the digester is expected to be loaded more than its

rated capacity. Similarly, retention days could be reduced if the temperature could be raised (KVIC, 1988). Temperature at 35°C enables the digestion process increased so that, the retention days can be shorter than ambient temperature retention days.

1.4. Contribution to the creation of safe manure from the digested manure

### 1.5. Specific objectives

To assess the factors of pH, C/N, and moisture of various microbial products in the effluent slurry of biogas digester tank as digesters

To investigate the possibility of pollution by untreated products of digester slurry

To recommend the safe management and utilization of the effluent slurry



### 3. OBJECTIVES OF THE STUDY

#### 3.1. General objective

- . To contribute to the creation of safe environment free from intestinal parasites

#### 3.2. Specific objectives

- . To assess the presence of eggs, cysts, and oocysts of various intestinal parasites in the effluent slurry of toilet linked biogas digesters
- . To examine the possible risk of pollution by intestinal parasites of the effluent slurry
- . To recommend better management and utilization of the effluent slurry





## 4. MATERIALS AND METHODS

### 4.1. Biogas digesters

The biogas digesters used for the assessment were fixed-dome shaped digesters, which were constructed with 3 to 1 ratios (digester volume to gas holder volume) in Addis Ababa, Fitcha and Awassa. The influent slurry of digesters was a human excreta.

### 4.2. Study areas and population

The digesters in Addis Ababa are located at Asko, Birsate-Gebriel and Lideta at altitude of 2555, 2300 and 2347 meters a. s. l. respectively. Digesters at Asko and Lideta, each had a volume of 22m<sup>3</sup> and the volume of that of Birsate-Gebriel was 25m<sup>3</sup>. All the digesters were linked with toilets. The toilets have been giving service for more than 25 households or 150 individuals each for Asko and Lideta, and 30 households or 180 individuals at Birsate-Gebriel for the last two years.

The digester at Fitcha, at elevation of 2800 meters a. s. l. and 120km from Addis Ababa to the northwest part of the country, had a volume of 8m<sup>3</sup>. The digester was linked with a toilet that has been giving service for three years for more than 70 individuals per day.

The digester at Awassa, at elevation of 1680 meters a. s. l. and 278km from Addis Ababa to the southern part of the country, had a volume of 25m<sup>3</sup>. The digester was linked with a toilet that has been giving service for two years for more than 30 households or 180 individuals.

### 4.3. Sample collection

Samples were collected four times in different seasons, that is, in *Tseday* (September to November 2004), *Bega* (December to February 2004/05), *Belg* (March to May 2005) and *Kiremt* (June to August 2005), as temperature varies from season to season. Collection was achieved when the influent slurry is channeled into the digester through mixing pit and the effluent slurry is released and channeled away through slurry reservoir. Each sample (50ml) taken before and

after digestion and transported to the laboratory of Akililu Lemma Institute of Pathobiology using icebox. Duration of the study was from 2004 *Tseday* to 2005 *Kiremt*.

Intervals between sampling season were determined taking into account the retention time of each digester at different season and at different site depending on the flow rate of the influent slurry. For this end, retention time was determined dividing the digester working volume by daily in put volume: i.e., Retention time (days) = Digester Volume ( $m^3$ ) / Daily in put Volume ( $m^3$ )

#### **4.4. Measurements**

##### *4.4.1. pH*

Acidity and alkalinity of both the influent slurry and effluent slurry of each digester was measured using pH meter for each season.

##### *4.4.2. Flow rate*

In order to determine retention time, the amount of influent slurry that entered into the digester was measured at the mixing pit in cubic meter throughout the seasons.

##### *4.4.3. Temperature*

Temperature of all digesters and the ambient temperature of each particular site were measured using digital thermometer for each season. The mean maximum and the mean minimum of the ambient and digesters temperature were indicated after measuring twice per day (at 6 AM and 12 AM) continuously for each season.

##### *4.4.4. Total solid*

In order to determine total solid (material left after evaporation of moisture) of all influent slurries, according to Standard Method of American Public Health Association (1997), samples (5ml) were dried in an oven at  $105^{\circ}C$  until a constant weight was obtained through repeated cycle of drying and cooling. Finally, the total solid was expressed in percent:

$TS\% = (A-B/A) \times 100$ : Where  
A = Evaporated moisture + Dried residue  
B = Evaporated moisture

#### 4.4.5. Free Ammonia

Free ammonia of samples of selected sites, that is, Fitcha, Awassa and Addis Ababa (Bisrate-Gebriel) was measured according to Nessler method (Hach, 1999). In line with this, the prepared samples (effluent and influent slurries) diluted with distilled water until they became within the range to be read by the spectrophotometer. Then, three drops of each Mineral Stabilizer and Dispersing Agent were added on 25ml of the diluted samples and inverted several times to mix. Finally, after adding three drops of Nessler Reagent and inverting, the samples were placed into the cell holder and the amount of free ammonia in milligram per liter was read. This value was converted into actual value with respect to the dilution process.

#### 4.5. Identification and enumeration of intestinal parasite

Formalin-ether concentration method and Ziehl Neelsen stained smear was applied to investigate fecal parasites. Formaline-ether concentration method was used to examine eggs of intestinal helminthes as well as cysts of intestinal protozoa. For this purpose, 1 ml of the influent and effluent slurry was diluted, sieved and eventually collected in centrifuge tube using 4ml of 10% formaline. After adding another 3ml of 10% formaline and 3ml ether, suspension was well mixed by shaking then centrifuged at 2000 revolution per minute for 5 minutes, the sediment was transferred to a slide. Examination was conducted using 10x and 40 x objectives to examine small cysts and eggs. To assist identification of cysts small drops of iodine were used under the cover glass. Eggs were counted and calculated per milliliter (ml) after observing all sediments.

To examine *Cryptosporidium* oocysts, a thin smear of the influent and effluent slurry was prepared fixed with methanol for 5 minutes, the slide flooded with carbol fuchsin and stained for 30 minutes at room temperature. Then the slide decolorized with acid alcohol after washing using tap water gently. It was counter stained with malachite green for 5 minutes. Then, washed with water, after drying, it was examined under oil immersion for the presence or absence of *Cryptosporidium* oocysts.

#### 4.6. Statistical analyses

The percentage of egg counts in the influent slurry and effluent slurry was used to evaluate the removal of eggs at the five sites (digesters). Data were analyzed using SPSS version 14.0. Comparing proportions (percentages) was useful to compare the removal of eggs with respect to seasons, sites and species using Z test for proportions. Correlation coefficient (r) was calculated between free ammonia concentration level of the effluent slurry and measured variables and between measured variables and elimination of *A. lumbricoides*, *T. trichiura* and *T. saginata*. Differences reported were significant at different significant level ( $P < 0.05$ ).

Table 1. Data collected from the different species.

SITE	WINTER		SUMMER		RAIN		TOTAL	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
ABATA	4.76	3.41	7.73	7.32	8.5	7.13	8.83	7.47
YANTA	4.33	7.77	3.42	7.45	5.25	7.31	6.33	5.56
BARATA	7.34	7.4	6.7	7.47	6.7	6.8	6.7	7.34
BARATA								
BARATA	4.27	7.1	6.7	7.38	7.1	6.34	6.42	7.01
BARATA	6.11	7.7	4.38	7.64	7.63	7.7	6.75	7.2



## 5. RESULTS

### 5.1. Monitoring of digesters

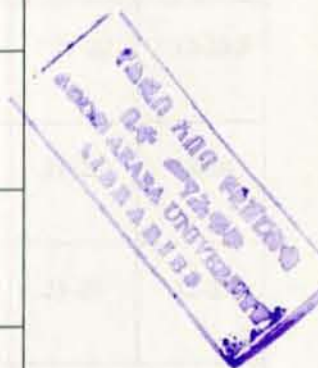
Characteristics of influent slurries and factors that affect the digestion process and characteristics of effluent slurries that affect the survival of parasites were assessed during monitoring. pH, retention days, temperature of digesters, total solids and free ammonia measured are indicated in Table 1, 2,3,4 and 5 respectively.

**Table 1. pH of influent and effluent slurries**

SITES	SEASONS							
	KIREMT		TSEDAY		BEGA		BELG	
	INF.	EFF.	INF.	EFF.	INF.	EFF.	INF.	EFF.
ASKO	6.76	7.45	6.72	7.52	6.6	7.63	6.83	7.47
LIDETA	6.55	7.37	6.88	7.44	6.65	7.61	6.78	7.56
BISRATE- GEBRIEL	7.14	7.4	6.58	7.47	6.7	7.65	6.95	7.59
FITCHE	6.69	7.1	6.7	7.38	7.1	7.54	6.82	7.41
AWASSA	6.71	7.5	6.79	7.64	6.62	7.7	6.84	7.6

**Table 2. Retention days of influent slurries in the digesters**

SITES	SEASONS			
	KIREMT	TSEDAY	BEGA	BELG
ASKO	77	74	67	70
LIDETA	75	71	64	67
BISRATE - GEBRIEL	82	76	68	73
FITCHE	87	80	74	79
AWASSA	75	70	61	68



**Table 3. Temperature of sites and digesters in degree Celsius**

SITES	SEASONS							
	KIREMT		TSEDAY		BEGA		BELG	
	DIGESTER	SITE	DIGESTER	SITE	DIGESTER	SITE	DIGESTER	SITE
ASKO	15-21	11.3-21	20-21	8-21	21.5-23	10-24.4	21-22	12-23.5
LIDETA	17-21.5	12-22	20-22	8-23	22-23	10-25	21-22	12-24
BISRATE- GEBRIEL	17-22	12-23	20-22.7	10-23	22-24	12-26	21-23	12-25
FITCHE	12-18	9-19	15-20	6-21	18-22	8-22	18-20	10-21
AWASSA	20-23	14-25	22-24	11-27	23-26	12-29	22-25	14-27

**Table 4. Total solids of influent slurry samples in percent**

SITES	SEASONS			
	KIREMT	TSEDAY	BEGA	BELG
ASKO	14.87	14.95	13	14.6
LIDETA	14	14.75	14.89	13.4
BISRATE - GEBRIEL	12.7	13.7	12.24	13.77
FITCHE	10.65	11	10.1	10.7
AWASSA	11.5	11.8	12.6	12.3



Coefficient of correlation, between the ammonia concentration level in the effluent slurry and average slurry temperature, at  $\alpha = 0.05$  showed that slurry temperature was significantly correlated with the ammonia concentration level in the slurry ( $r = 0.01$ ). However,  $r^2$  of effluent slurry at  $\alpha = 0.01$  revealed that  $r = 0.94$  and  $r^2 = 0.88$  and we can show significant positive correlation with the ammonia concentration level in the

**Table 5. Free ammonia concentration level of selected samples of influent and effluent slurries with respect to measured variables**

SITE	SEASON	MEASURED VARIABLES				NH <sub>3</sub> (mg/liter)	
		pH OF INF. SLURRY	TOTAL SOLID (%)	RETENTION TIME (DAYS)	AVERAGE TEMPERATURE OF DIGESTER (°C)	INFLUENT SLURRY	EFFLUENT SLURRY
FITCHE	KIREMT	6.69	10.65	87	15	490	780
FITCHE	BEGA	7.1	10.1	74	20	570	1180
BISRATE-GEBRIEL	KIREMT	7.14	12.7	82	19.5	570	1000
BISRATE-GEBRIEL	BEGA	6.7	12.24	68	23	610	1250
AWASSA	KIREMT	6.71	11.5	75	21.5	540	1220
AWASSA	BEGA	6.62	12.6	71	24.5	640	1360

Coefficient of correlation, between free ammonia concentration level in the effluent slurry and average digester temperature, ( $r = 0.96$ ) showed that digester temperature was significantly correlated with free ammonia concentration level in the digester ( $P < 0.01$ ). However, pH of influent slurry ( $r = -0.22$ ), retention time ( $r = -0.94$ ) and total solid ( $r = 0.36$ ) did not show significant positive correlation with free ammonia concentration level ( $P > 0.05$ ).

**Table 6. Correlation coefficient (r) between measured variables and elimination of *A. lumbricoides*, *T. trichiura* and *T. saginata* regardless of seasons and sites**

Variables	Correlation coefficient (r)		
	<i>A. lumbricoides</i>	<i>T. trichiura</i>	<i>T. saginata</i>
pH	- 0.27	-0.29	-0.36
Retention time	- 0.68	-0.81	- 0.8
Temperature	0.83	0.88	0.84
Total solids	0.24	0.1	0.31

The correlation coefficient between the variables and elimination of parasites indicated that temperature was the variable that showed significant positive correlation with elimination of *A. lumbricoides*, *T. saginata* ( $P < 0.001$ ) and *T. trichiura* ( $P < 0.01$ ) (Table 6). On the other hand, retention time, total solid and pH did not show significant positive correlation ( $P > 0.05$ ) with elimination. However, retention time showed significant ( $P < 0.01$ ) negative correlation with the parasites.

## 5.2. Presence of eggs of intestinal parasites in the samples

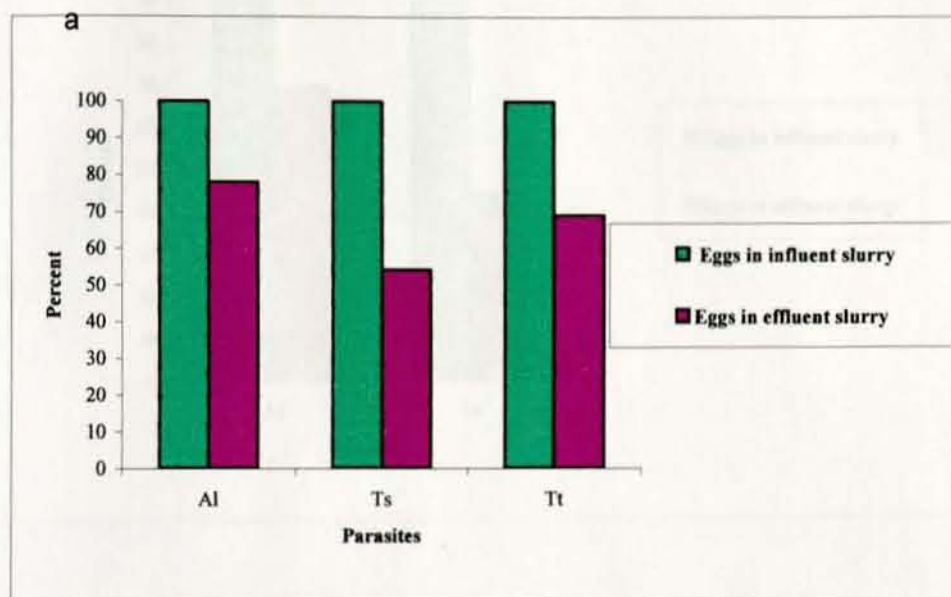
Eggs of different species of intestinal helminthes were found in the influent slurry of all digesters in all seasons. The arithmetic mean of number of eggs per ml per season was calculated to be, 18.13, 4.37 and 7.3 for Asko digester, 16.37, 6.47 and 5.26 for Lideta digester, 11.1, 8.4 and 5.3 for Bistrate-Gebriel digester, 9.15, 14.55 and 3.9 for Fitcha digester and 14.75, 31.25 and 8.9 for Awassa digester for *Ascaris lumbricoides*, *Taenia saginata* and *Trichuris trichiura* respectively. Eggs of *F. hepatica* (from Bistrate-Gebriel and Awassa digesters) and *S. mansoni* (from Awassa digester) were also detected in the influent slurry (4 eggs per ml of each species). The overall elimination of these eggs in the digesters presented in each site and season. Figures 1a-d, 2a-d, 3a-d, 4a-d and 5a-d show percentage of egg counts per ml in the influent and effluent slurry of Asko, Lideta, Bistrate-Gebriel, Fitcha and Awassa digesters respectively.



Figure 1a. Percentage of egg counts per ml in the influent and effluent slurry for *Ascaris lumbricoides* (a), *T. saginata* (b) and *T. trichiura* (c) during period 2005-06 (Asko digester).

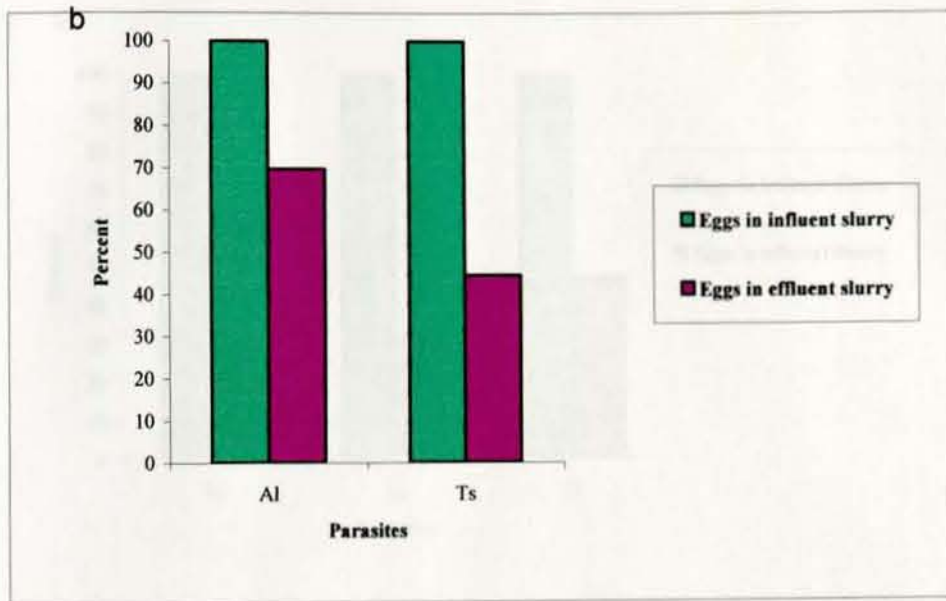
In period 2005-06 eggs of *A. lumbricoides*, 52.4% of *T. saginata* and 60% of *T. trichiura* were detected in the effluent slurry from Asko (Figure 1a). Significantly reduced egg counts were observed in the effluent slurry compared to the influent slurry for *T. saginata* ( $P < 0.001$ ) but not for *T. trichiura* ( $P < 0.05$ ). Similarly, there was significant difference between *T. trichiura* and *T. saginata* ( $P < 0.05$ ).

### 5.2.1. Asko



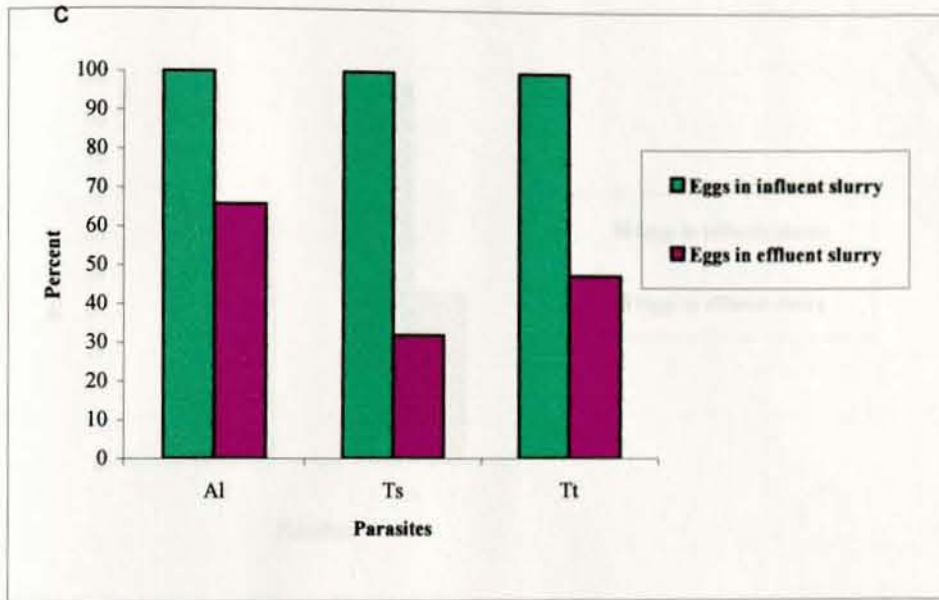
**Figure 1a.** Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts) and *T. trichiura* (Tt) during Kiremt 2005 at Asko digester.

In Kiremt, 78% of eggs of *A. lumbricoides*, 52.4% of *T. saginata* and 69% of *T. trichiura* were detected in the effluent slurry from Asko (Figure 1a). Regarding statistical difference among species, *A. lumbricoides* was significantly different from *T. saginata* ( $P < 0.001$ ) but not from *T. trichiura* ( $P > 0.05$ ). Similarly, there was significant difference between *T. trichiura* and *T. saginata* ( $P < 0.05$ ).



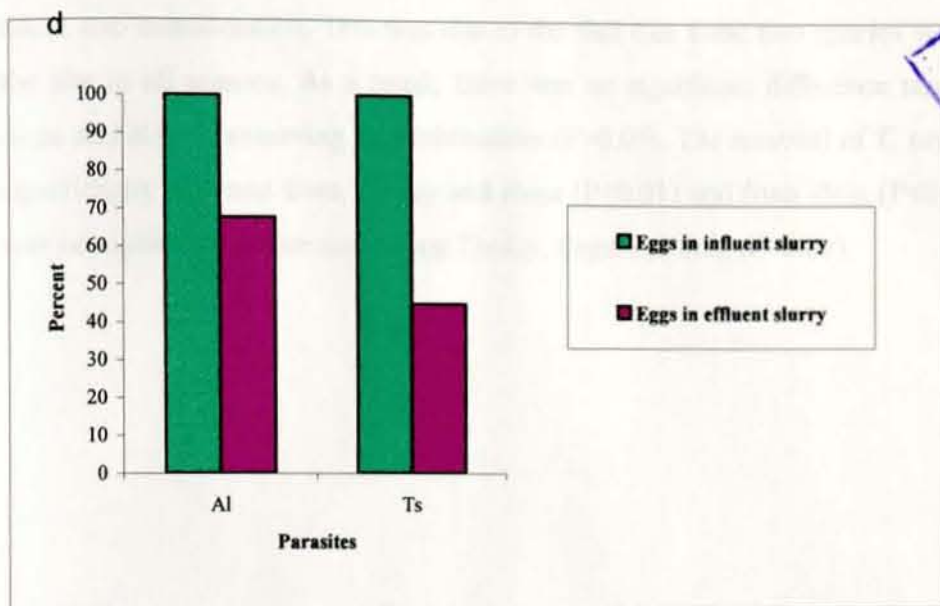
**Figure 1b. Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al) and *T. saginata* (Ts) during Tseday 2004 at Asko.**

In Tseday, the removal of percentage was 69.7 and 44.5 for *A. lumbricoides* and *T. saginata* in the effluent slurry respectively (Figure 1b). As a result, *A. lumbricoides* showed significant difference from *T. saginata* ( $P < 0.001$ ).



**Figure 1c.** Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts) and *T. trichiura* (Tt) during Bega 2004/05 at Asko.

In Bega, 65.8%, 47.6% and 32.1% of eggs of *A. lumbricoides*, *T. trichiura* and *T. saginata* respectively were recorded in the effluent slurry (Figure 1c). Concerning difference among species, the elimination of eggs of *A. lumbricoides* was significantly different from *T. saginata* ( $P < 0.001$ ) and *T. trichiura* ( $P < 0.01$ ). Similarly, there was also significant difference between these latter species ( $P < 0.05$ ).



**Figure 1d. Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al) and *T. saginata* (Ts) during Belg 2005 at Asko.**

In Belg, 67.8% of eggs of *A. lumbricoides* and 45% of *T. saginata*, were recorded in the effluent slurry (Figure 1d). The removal of *A. lumbricoides* was significantly different from *T. saginata* ( $P < 0.01$ ).

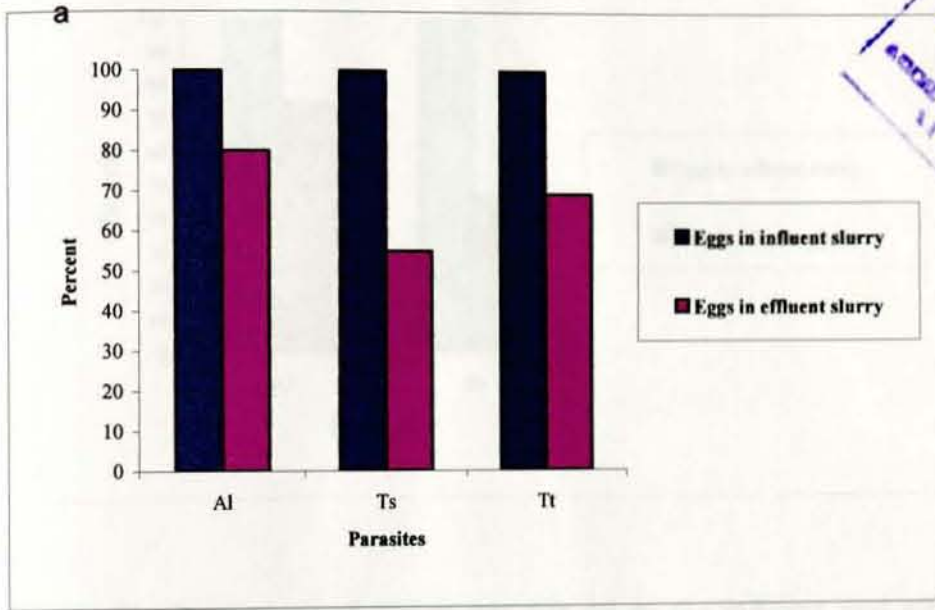
In order to evaluate removal difference across seasons, *A. lumbricoides* and *T. saginata* was taken into consideration. This was due to the fact that these two species were being detected at the site in all seasons. As a result, there was no significant difference among *Kiremt*, *Tseday*, *Bega* and *Belg* in removing *A. lumbricoides* ( $P > 0.05$ ). The removal of *T. saginata* in *Kiremt* was significantly different from *Tseday* and *Bega* ( $P < 0.01$ ) and from *Belg* ( $P < 0.05$ ). However, there was no significant difference among *Tseday*, *Bega* and *Belg* ( $P > 0.05$ ).



Figure 2a. Percentage of egg removal per all in the different well affected slurry for *A. lumbricoides* (A), *T. saginata* (B) and *E. coli* (C) during period 2005 in Laliba.

The different slurry of Laliba digestion of the slurry (Figure 2) showed 10%, 80.2% and 10% of eggs of *A. lumbricoides*, *T. saginata* and *E. coli* respectively (Figure 2). As a result, *A. lumbricoides* showed significant difference from *T. saginata* ( $P < 0.001$ ) but not from *E. coli* ( $P > 0.05$ ). Similarly, significant difference was observed between *T. saginata* and *E. coli* ( $P < 0.05$ ).

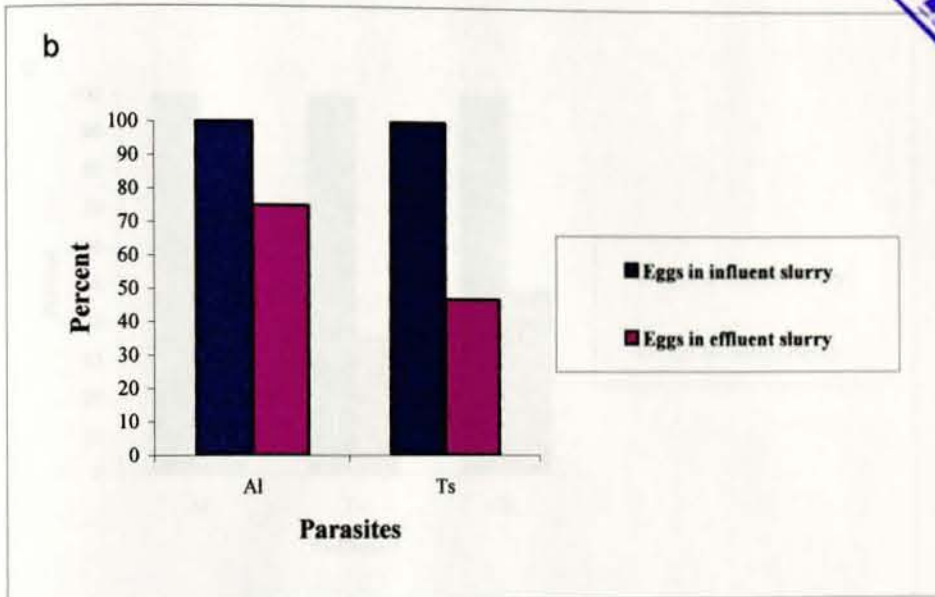
5.2.2. Lideta



**Figure 2a.** Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts) and *T. trichiura* (Tt) during Kiremt 2005 at Lideta.

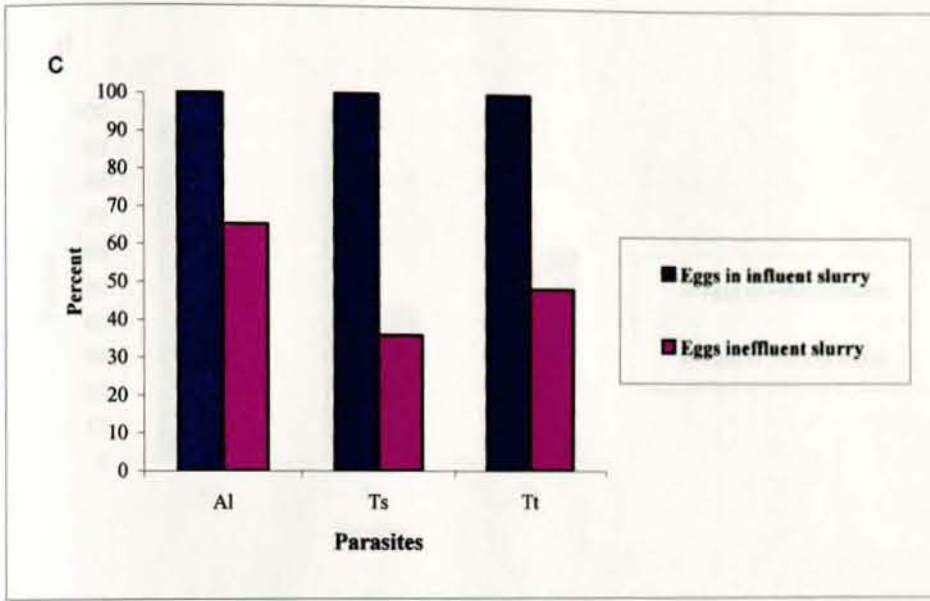
The effluent slurry of Kiremt digestion of the biogas digester at Lideta showed 80%, 69.2% and 55% of eggs of *A. lumbricoides*, *T. trichiura* and *T. saginata* respectively (Figure 2a). As a result, *A. lumbricoides* showed significant difference from *T. saginata* ( $P < 0.001$ ) but not from *T. trichiura* ( $P > 0.05$ ). Similarly, significant difference was observed between *T. trichiura* and *T. saginata* ( $P < 0.05$ ).

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አዲስ አበባ ዩኒቨርሲቲ  
ኢንፎርሜሽን ቴክኖሎጂ



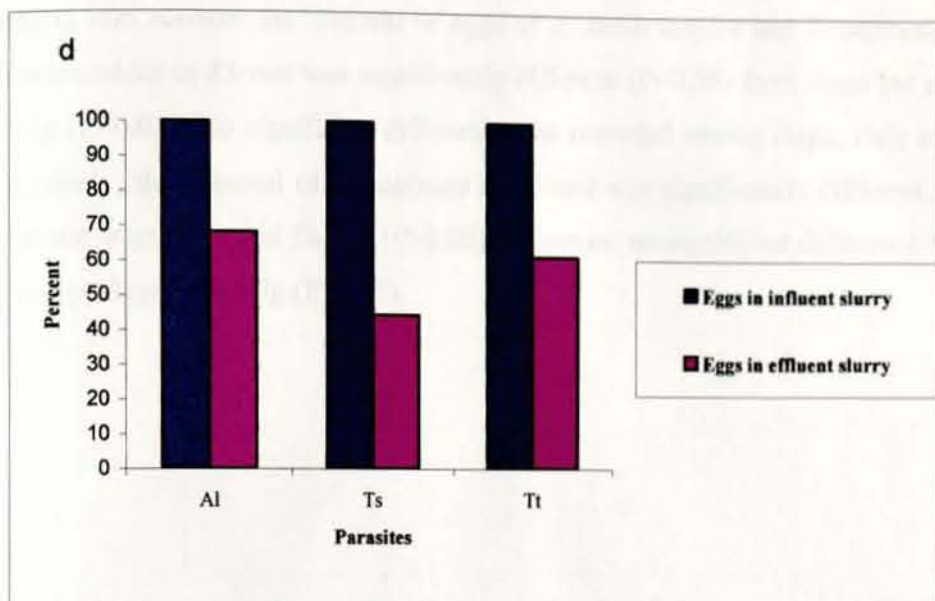
**Figure 2b.** Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al) and *T. saginata* (Ts) during Tseday 2004 at Lideta.

After Tseday digestion, 75% of eggs of *A. lumbricoides* and 47% of eggs of *T. saginata* found in the effluent slurry (Figure 2b). Significant difference was found between *A. lumbricoides* and *T. saginata* ( $P < 0.001$ ).



**Figure 2c.** Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts) and *T. trichiura* (Tt) during Bega 2004/05 at Lideta.

In Bega, 65.3%, 36% and 48.4% of eggs of *A. lumbricoides*, *T. saginata* and *T. trichiura* were observed respectively during examination of the effluent slurry (Figure 2c). Significant difference was found between *A. lumbricoides* and *T. saginata* ( $P < 0.001$ ). Similarly, there was significant difference between *A. lumbricoides* and *T. trichiura* ( $P < 0.05$ ). However, there was no significant difference between *T. saginata* and *T. trichiura* ( $P > 0.05$ ).



**Figure 2d. Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts) and *T. trichiura* (Tt) during Belg 2005 at Lideta.**

In Belg, 68.2% of eggs of *A. lumbricoides*, 61.5% of *T. trichiura* and 44.5% of *T. saginata* remained in the effluent slurry (Figure 2d). Significant difference was observed between *A. lumbricoides* and *T. saginata* ( $P < 0.001$ ) and between *T. saginata* and *T. trichiura* ( $P < 0.05$ ). However, there was no significant difference between *A. lumbricoides* and *T. trichiura* ( $P > 0.05$ ).

In another development, in evaluating the seasonal difference in removing eggs from the digester, taking into account the removal of eggs of *A. lumbricoides* and *T. saginata*, the removal of *A. lumbricoides* in *Kiremt* was significantly different ( $P < 0.05$ ) from *Bega* but not from *Tseday* and *Belg* ( $P > 0.05$ ). No significant difference was recorded among *Bega*, *Belg* and *Tseday* ( $P > 0.05$ ). Similarly, the removal of *T. saginata* in *Kiremt* was significantly different ( $P < 0.01$ ) from *Bega* but not from *Belg* and *Tseday* ( $P > 0.05$ ). However, no significant difference was recorded among *Tseday*, *Bega* and *Belg* ( $P > 0.05$ ).



Figure 26. Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (A), *T. saginata* (B) and *T. evansi* (C) during Kiremt (2009), Bega (2010) and Belg (2011).

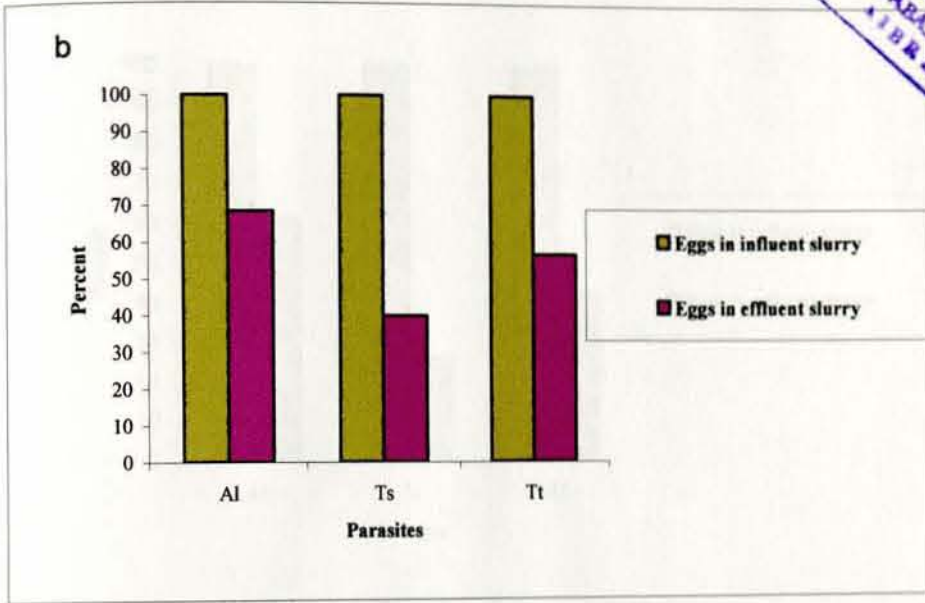
When the digester process, 74% of eggs of *A. lumbricoides*, 87% of *T. saginata* and 100% of *T. evansi* were removed in the effluent slurry from Fildes (Table 16) during Kiremt (Figure 26). Regarding differences in egg counts per ml removal process among seasons, significant difference was found between *A. lumbricoides* and *T. saginata* ( $P < 0.01$ ) and between *T. evansi* and *T. saginata* ( $P < 0.05$ ). However, there was no significant difference between *A. lumbricoides* and *T. evansi* ( $P > 0.05$ ).



**Figure 3a.** Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts) and *T. trichiura* (Tt) during Kiremt 2005 at Bisrate - Gebriel.

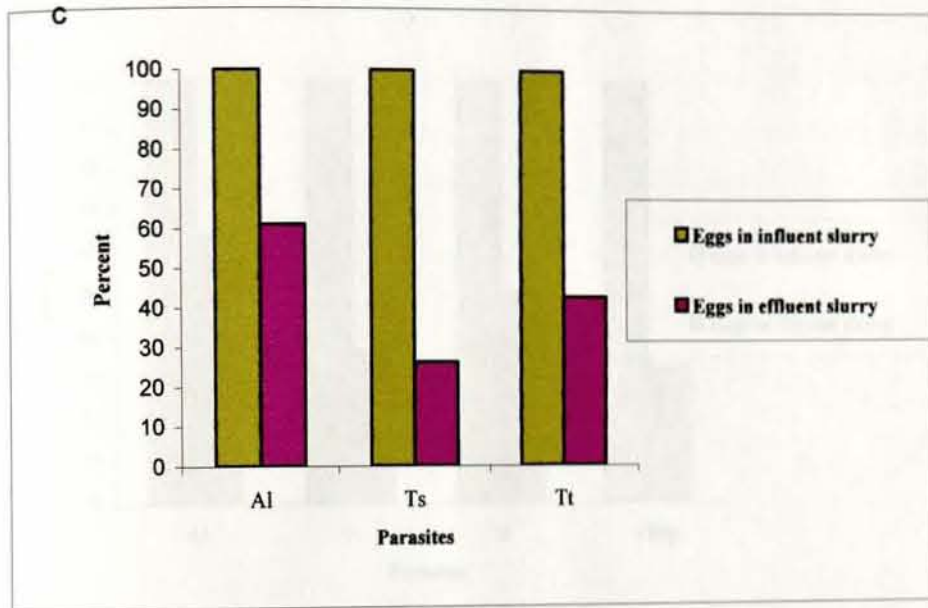
After the digestion process, 74% of eggs of *A. lumbricoides*, 69% of *T. trichiura* and 54.5% of *T. saginata* remained in the effluent slurry from Bisrate-Gebriel in Kiremt (Figure 3a). Regarding difference in withstanding the removal process among species, significant difference was found between *A. lumbricoides* and *T. saginata* ( $P < 0.01$ ) and between *T. trichiura* and *T. saginata* ( $P < 0.05$ ). However, there was no significant difference between *A. lumbricoides* and *T. trichiura* ( $P > 0.05$ ).





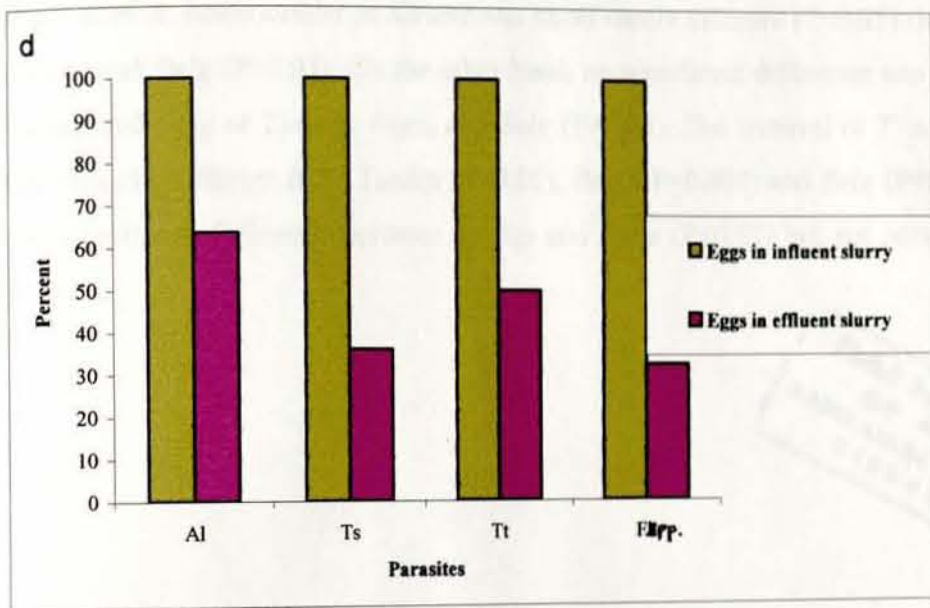
**Figure 3b. Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts) and *T. trichiura* (Tt) during Tseday 2004 at Bisrate - Gebriel.**

In Tseday, after digestion of the season, 68.5% of *A. lumbricoides*, 40% of *T. saginata* and 56.8% of *T. trichiura* were observed in the effluent slurry (Figure 3b). Significant difference was observed between *A. lumbricoides* and *T. saginata* ( $P < 0.001$ ) and between *T. trichiura* and *T. saginata* ( $P < 0.05$ ).



**Figure 3c. Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts) and *T. trichiura* (Tt) during Bega 2004/05 at Bisrate - Gebriel.**

Concerning eggs in *Bega* digestion, 61.1% of *A. lumbricoides*, 42.8% of *T. trichiura* and 26.3% of *T. saginata* were remained in the effluent slurry (Figure 3c). Significant difference was observed between *A. lumbricoides* and *T. saginata* ( $P < 0.001$ ) and between *T. trichiura* and *T. saginata* ( $P < 0.05$ ). Similarly, significant difference ( $P < 0.01$ ) was recorded between *A. lumbricoides* and *T. trichiura*.



**Figure 3d. Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts), *T. trichiura* (Tt) and *Fasciola* species (Fsp) during Belg 2005 at Bisrate - Gebriel.**

After Belg digestion, 63.6%, 50%, 36% and 32.5% of eggs of *A. lumbricoides*, *T. trichiura*, *T. saginata* and *Fasciola* species indicated respectively in the effluent slurry (Figure 3d). However there was no significant ( $P>0.05$ ) difference between these two parasites. Significant difference was also found between *A. lumbricoides* and *T. trichiura*, between *T. trichiura* and *T. saginata* ( $P<0.05$ ) and between *T. trichiura* and *Fasciola* species ( $P<0.01$ ). In the same manner, there was significant difference between *A. lumbricoides* and *T. saginata* and between *A. lumbricoides* and *Fasciola* species ( $P<0.001$ ). However, there was no significant difference between *T. saginata* and *Fasciola* species ( $P>0.05$ ).

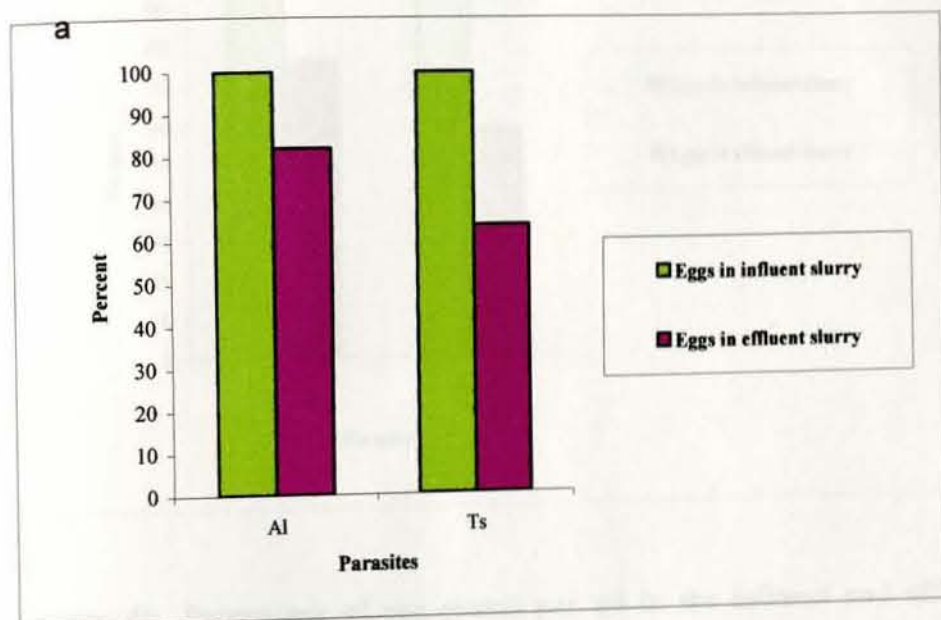
Concerning difference across seasons, based on the removal of *A. lumbricoides* and *T. saginata*, the removal of *A. lumbricoides* in *Kiremt* was significantly different ( $P < 0.05$ ) from *Bega* but not from *Tseday* and *Belg* ( $P > 0.05$ ). On the other hand, no significant difference was found among *Kiremt*, *Tseday* and *Belg* or *Tseday*, *Bega*, and *Belg* ( $P > 0.05$ ). The removal of *T. saginata* in *Kiremt* was significantly different from *Tseday* ( $P < 0.05$ ), *Bega* ( $P < 0.001$ ) and *Belg* ( $P < 0.01$ ). Similarly, there was significant difference between *Tseday* and *Bega* ( $P < 0.05$ ) but not between *Tseday* and *Belg* ( $P > 0.05$ ).



Figure 10. Percentage of egg count per 100 g soil samples and different sites for *A. lumbricoides* (A) and *T. saginata* (B) during Kiremt 2007 in Ethiopia.

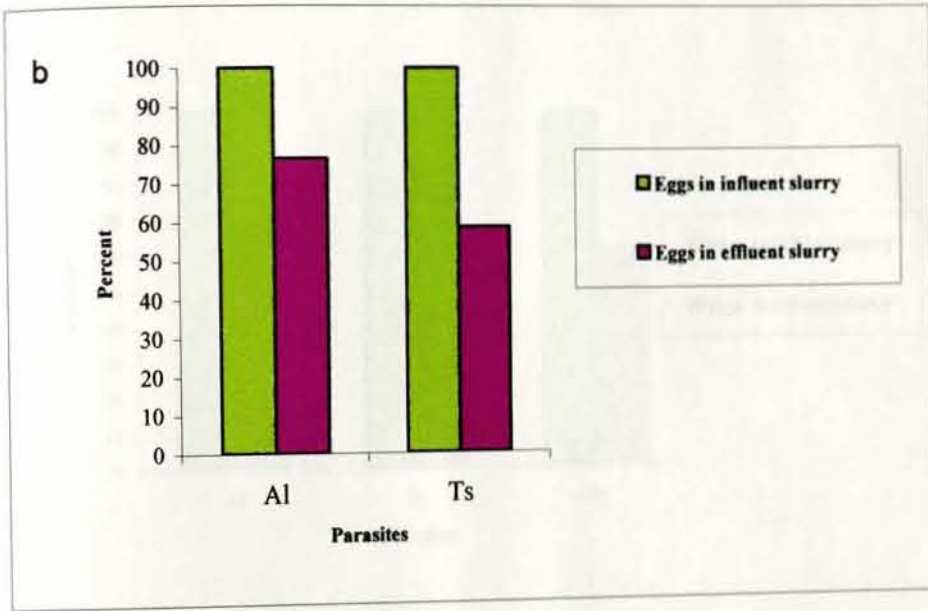
Overall number of species recorded in the collection of animal and different sites of the study was the lowest in Kiremt season. During this time, in Kiremt season, eggs of *A. lumbricoides* (10%) and *T. saginata* (15.0%) were observed and significant difference in percentage difference between species that was significant difference in percentage composition between *A. lumbricoides* and *T. saginata* ( $P < 0.01$ ).

#### 5.2.4. Fitch



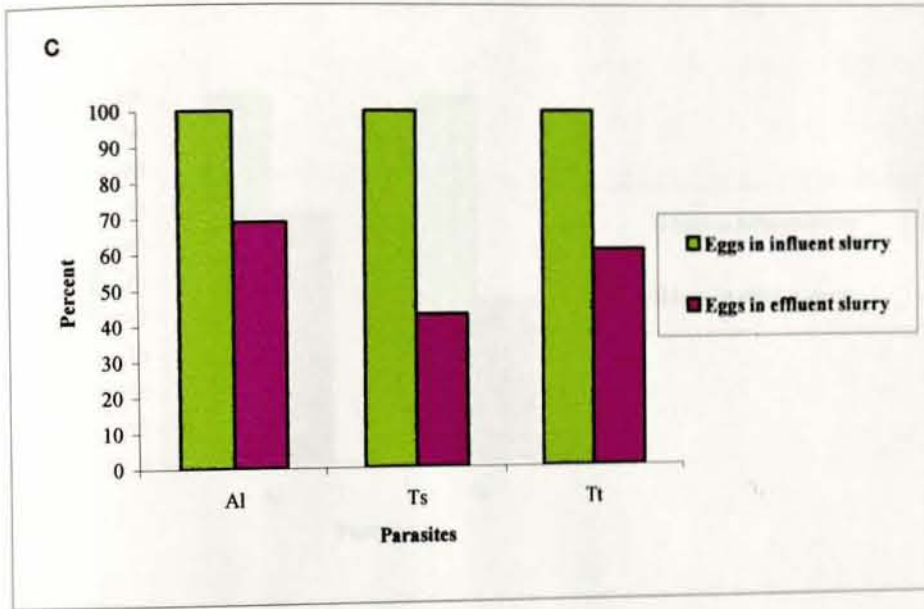
**Figure 4a.** Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al) and *T. saginata* (Ts) during Kiremt 2005 at Fitch.

The total number of species recorded in the influent slurry and effluent slurry of Fitch digester was the lowest as compared samples from other sites. In Kiremt digestion, eggs of *A. lumbricoides* (82%) and *T. saginata* (63.6%) were observed after digestion (Figure 4a). Concerning difference between species, there was significant difference in percentage (proportion) between *A. lumbricoides* and *T. saginata* ( $P < 0.01$ ).



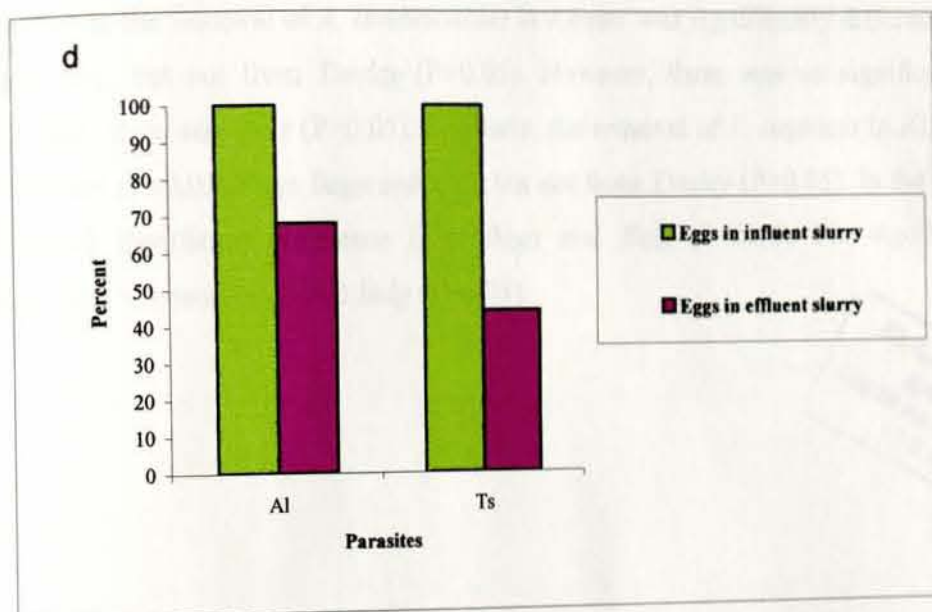
**Figure 4b. Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al) and *T. saginata* (Ts) during Tseday 2004 at Fitch.**

In Tseday, 76.6% of *A. lumbricoides* and 58.75% of *T. saginata* remained in the effluent slurry (Figure 4b). Concerning difference between species, there was significant difference in percentage (proportion) between *A. lumbricoides* and *T. saginata* ( $P < 0.01$ ).



**Figure 4c. Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts) and *T. trichiura* (Tt) during Bega 2004/05 at Lideta.**

In Bega digestion, 69%, 61% and 43% of eggs of *A. lumbricoides*, *T. trichiura* and *T. saginata* respectively remained in the effluent slurry (Figure 4c). As a result, *A. lumbricoides* showed significant difference from *T. saginata* ( $P < 0.001$ ) but not from *T. trichiura* ( $P > 0.05$ ). However, *T. trichiura* showed significant difference from *T. saginata* ( $P < 0.05$ ).



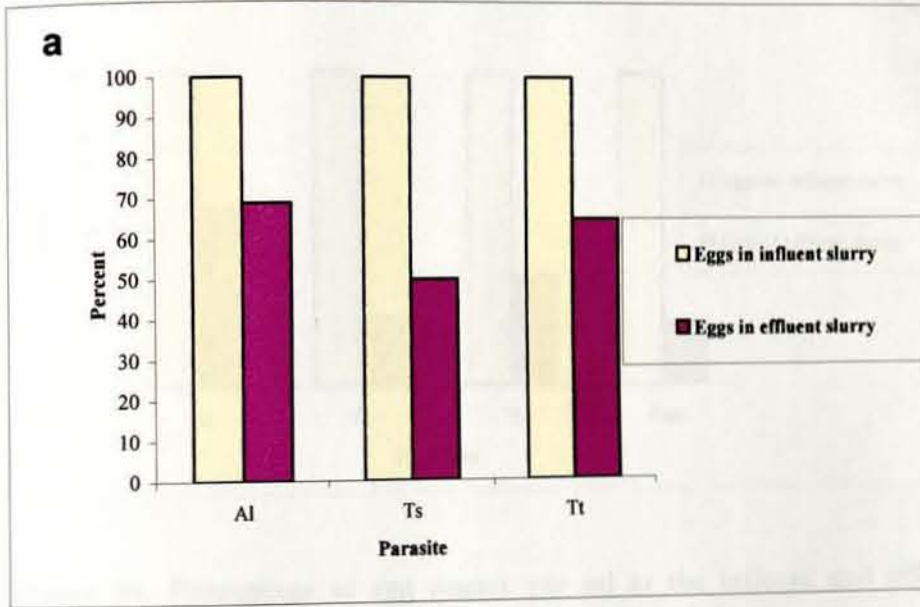
**Figure 4d. Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al) and *T. saginata* (Ts) during Belg.**

In the same manner, in Belg, 68.7% of *A. lumbricoides* and 44.5% of *T. saginata* were recorded in the effluent slurry (Figure 4d). Concerning difference between species, there was significant difference in percentage (proportion) between *A. lumbricoides* and *T. saginata* ( $P < 0.01$ ).

Regarding difference across seasons, with respect to the removal of *A. lumbricoides* and *T. saginata*, the removal of *A. lumbricoides* in *Kiremt* was significantly different ( $P < 0.05$ ) from *Bega* and *Belg* but not from *Tseday* ( $P > 0.05$ ). However, there was no significant difference among *Tseday*, *Bega* and *Belg* ( $P > 0.05$ ). Similarly, the removal of *T. saginata* in *Kiremt* was significantly different ( $P < 0.01$ ) from *Bega* and *Belg* but not from *Tseday* ( $P > 0.05$ ). In the same manner, *Tseday* showed significant difference from *Bega* and *Belg* ( $P < 0.05$ ). No significant difference was recorded between *Bega* and *Belg* ( $P > 0.05$ ).



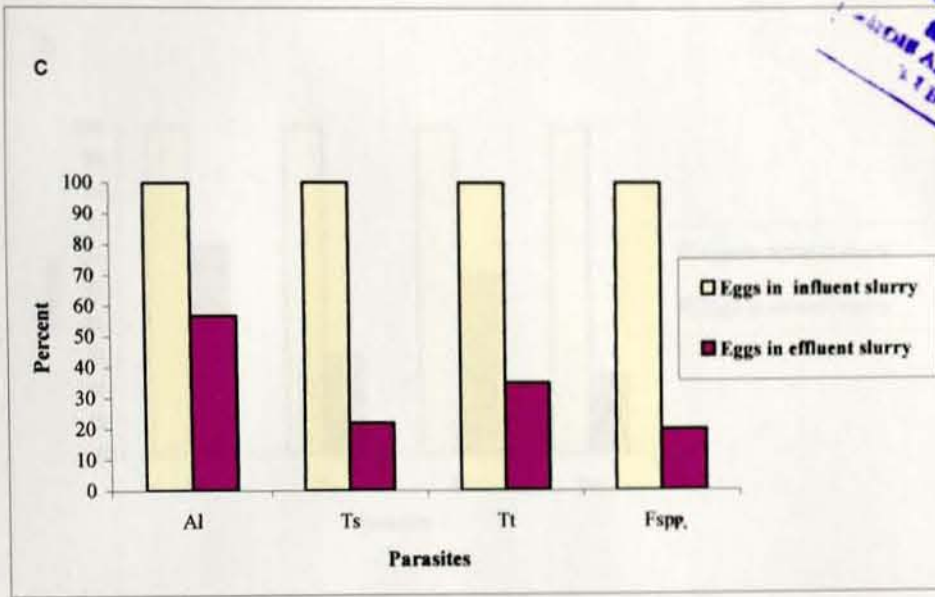
### 5.2.5. Awassa



**Figure 5a.** Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts) and *T. trichiura* (Tt) during Kiremt 2005 at Awassa.

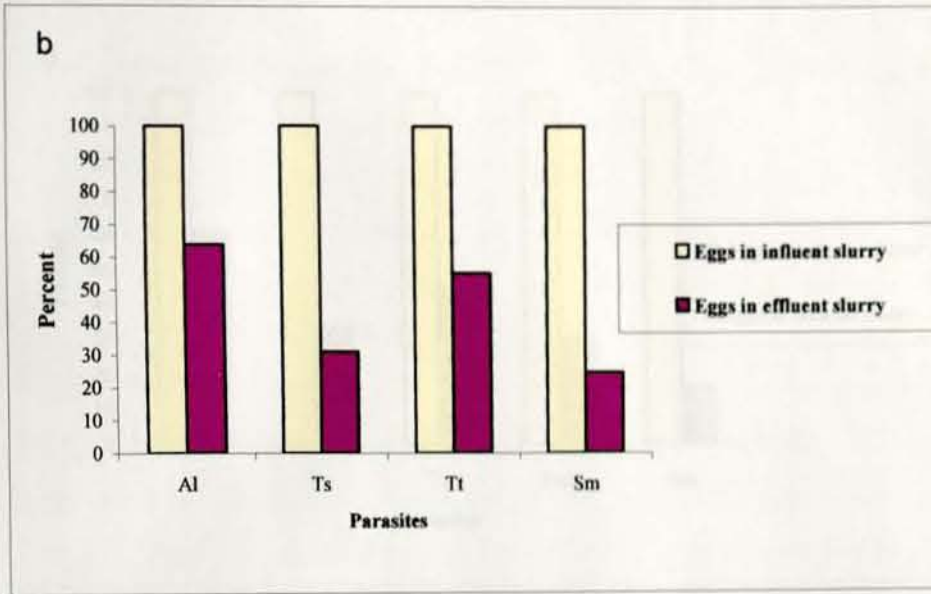
In Kiremt, 69% of *A. lumbricoides*, 64.8% of *T. trichiura* and 50% *T. saginata* were recorded in the effluent slurry (Figure 5a). Regarding difference among species in withstanding the Kiremt digestion process, significant difference was recorded between *A. lumbricoides* and *T. saginata* ( $P < 0.01$ ) and between *T. trichiura* and *T. saginata* ( $P < 0.05$ ). However, there was no significant difference between *A. lumbricoides* and *T. trichiura* ( $P > 0.05$ ).

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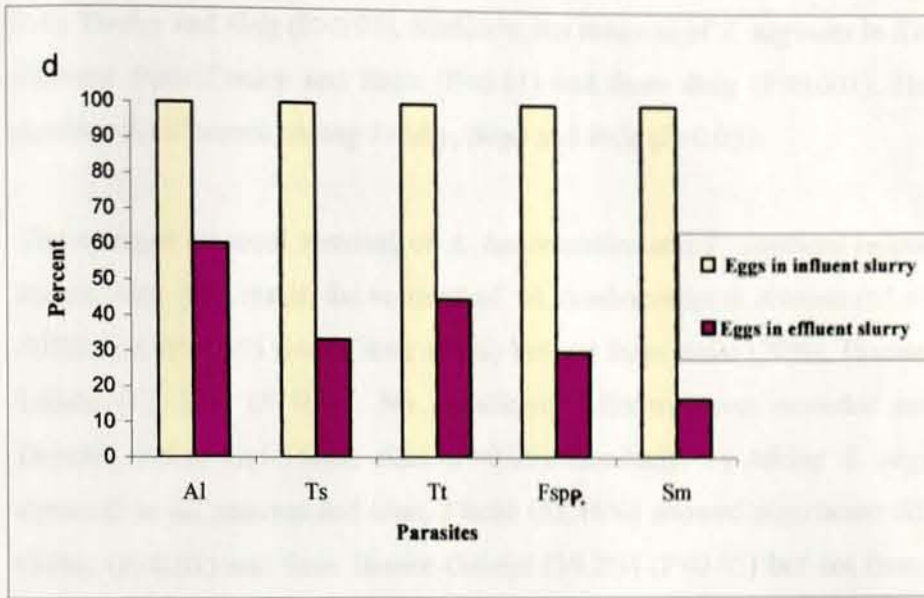
**Figure 5c.** Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts), *T. trichiura* (Tt) and *Fasciola* species (F spp) during Bega 2004/05 at Awassa.

In Bega, 56.8%, 35%, 20% and 22% of eggs of *A. lumbricoides*, *T. trichiura*, *Fasciola* species and *T. saginata* were recorded respectively (Figure 5c). As a result, *A. lumbricoides* showed significant difference from *Fasciola* species and *T. saginata* ( $P < 0.001$ ). Similarly, *T. trichiura* showed significant difference from *T. saginata* and *A. lumbricoides* ( $P < 0.05$ ). However, significant difference did not found between *T. saginata* and *Fasciola* species ( $P > 0.05$ ).



**Figure 5b. Percentage of egg counts per ml for in the influent and effluent slurry *A. lumbricoides* (Al), *T. saginata* (Ts), *T. trichiura* (Tt) and *S. mansoni* (Sm) during Tseday 2004/05 at Awassa.**

In Tseday, 63.8% of *A. lumbricoides*, 55% of *T. trichiura*, 31% of *T. saginata* and 24.7% *S. mansoni* were detected (Figure 5b). In line with this, there was significant difference between *A. lumbricoides* and *T. saginata*, between *T. trichiura* and *T. saginata*, between *A. lumbricoides* and *S. mansoni* and between *T. trichiura* and *S. mansoni* ( $P < 0.001$ ). However, there was no significant difference between *A. lumbricoides* and *T. trichiura* and between *T. saginata* and *S. mansoni* ( $P > 0.05$ )



**Figure 5d. Percentage of egg counts per ml in the influent and effluent slurry for *A. lumbricoides* (Al), *T. saginata* (Ts), *T. trichiura* (Tt), *Fasciola* species (F spp) and *S. mansoni* (Sm) during Belg 2005 at Awassa.**

In, Belg, 60.4% of *A. lumbricoides*, 44.9% of *T. trichiura*, 33.3% of *T. saginata*, 30% of *Fasciola* species and 16.7% of *S. mansoni* were observed in the effluent slurry respectively (Figure 5d). In line with this, *A. lumbricoides* showed significant difference from *T. saginata*, *Fasciola* species, *S. mansoni* ( $P < 0.001$ ) and *T. trichiura* ( $P < 0.05$ ). Similarly, *T. trichiura* showed significant difference from *Fasciola* species ( $P < 0.05$ ) and *S. mansoni* ( $P < 0.001$ ) but not from *T. saginata* ( $P > 0.05$ ). There was also significant difference between *T. saginata* and *S. mansoni* ( $P < 0.05$ ). However, there was no significant difference between *T. saginata* and *Fasciola* species ( $P > 0.05$ ). In the same manner, *Fasciola* species showed significant difference from *S. mansoni* ( $P < 0.05$ ).

The removal of *A. lumbricoides* in *Kiremt* was significantly different ( $P < 0.05$ ) from *Bega* but not from *Tseday* and *Belg* ( $P > 0.05$ ). Similarly, the removal of *T. saginata* in *Kiremt* was significantly different from *Tseday* and *Bega* ( $P < 0.01$ ) and from *Belg* ( $P < 0.001$ ). However, there was no significant difference among *Tseday*, *Bega* and *Belg* ( $P > 0.05$ ).

The average seasonal removal of *A. lumbricoides* and *T. saginata* helped to assess difference across sites. As a result, the removal of *A. lumbricoides* at Awassa (62.4%) showed significant difference ( $P < 0.05$ ) from Fitche (74%) but not from Asko (70%), Bistrate-Gebriel (66.8%) and Lideta (72.12%) ( $P > 0.05$ ). No significant difference was recorded among Fitche, Bistrate-Gebriel, Asko, and Lideta sites ( $P > 0.05$ ). Similarly, by taking *T. saginata* that was being detected in all seasons and sites, Fitche (52.46%) showed significant difference from Awassa (34%) ( $P < 0.01$ ) and from Bistrate-Gebriel (39.2%) ( $P < 0.05$ ) but not from Lideta (45.62%) and Asko (43.5%) ( $P > 0.05$ ). No significant difference was recorded among Awassa, Lideta, Bistrate-Gebriel and Asko ( $P > 0.05$ ).

In general, regardless of species and based on the average elimination per season, only less than fifty percent of the total recorded eggs were eliminated from each digester except Awassa digester, in which case almost sixty percent were removed.

### 5. 3. Protozoan parasites

A total of 12 positive slides of *Entamoeba* species were detected in the influent slurry of all digester at different seasons. In *Tseday* at Awassa, Bistrate-Gebriel and Fitche, in *Bega* at Asko, Bistrate-Gebriel, Fitche and Lideta, in *Kiremt* at Fitche and in *Belg* at Asko, Awassa, Fitche and Lideta digesters the positive slides of *Entamoeba* species was recorded. However, all slides of the effluent slurry were negative except that of Fitche in *Kiremt* and *Tseday*.

In another situation, *Cryptosporidium* species (4 to 5 microns in diameter) was found in samples from three digesters and in two seasons. Positive samples of the influent slurry and effluent slurry of Asko and Lideta digesters in *Tseday* and Awassa in *Bega* were detected.

## 6. DISCUSSION

The assessment of intestinal parasites in the effluent slurry of biogas digesters working with in the range of ambient temperature revealed the survival of eggs of various intestinal parasites such as *A. lumbricoles*, *T. saginata*, *T. trichiura*, *S. mansoni* and *Fasciola* species. This was due to significant positive correlation of temperature with the elimination of eggs. In addition the significant differences, in eliminating eggs, between *Kiremt* and *Bega* and between *Fitche* and *Awassa* digester temperature may explain the crucial role of temperature than other variables, that is, total solid, pH and retention time for the elimination of eggs. Feachem *et al.* (1978) noted that the degree of destruction of eggs appears to be closely related to temperature. The negative correlation between retention time and elimination may be due to the inverse relation ship between temperature and retention time. This is to say that, whenever temperature increases, the retention time becomes shorter. Mattock (1984) noted that the retention period could be considerably reduced if the temperature raised.

On the other hand, the ammonia content of effluent slurries increased after digestion. In line with this, significant positive correlation was found between temperature and free ammonia concentration level after digestion that varied from site to site and from season to season.

According to Mara (1980), temperature plays significant role for the increment of free ammonia, which create a conducive condition for elimination of eggs in general. However, according to this study, all eggs couldn't be eliminated from digesters even from those with highest digester temperature or free ammonia concentration level, such a, at *Awassa* in *Bega*. In this regard, with reference to indicator organism, *Ascaris lumbricoles*, only 43.2% could be removed at the above mentioned free ammonia concentration level. WHO (1980) indicated that there is almost removal of *Ascaris lumbricoles* by half percentage in a digester working with in ambient temperature range (10-29<sup>0</sup>C). China State Biogas Association (CSBA) (1985) also indicated all eggs of *Ascaris lumbricoles* couldn't be eliminated at free ammonia concentration level of 3600mg/litre but 93.7%.

Regarding difference in withstanding the removal process, *A. lumbricoles* was the most resistant species that contributed to the largest quantity of eggs in each season and digester. This feature could be attributed to its nature to resist the toxic substance. Mara (1983) mentioned that since

*Ascaris* has thick protective sheath, it has remarkable capacity to survive longer periods under severe conditions. Likewise, the prevalence of other parasites was large in quantity. In line with this, *T. trichiura* seemed to be the other resistant species next to *A. lumbricoides* since of the total cases that these species detected, around 80% percent showed marked but non-significant difference between them. Bitton (1999) revealed especially the survival of *A. lumbricoides* and *T. trichiura* in mesophilic digestion as compared to other eggs. In addition to these two parasites, *Taenia saginata* and *Fasciola* species per ml was higher as compared to *Schistosoma mansoni* that has the larger removal rate. Mattock (1984) also reported similar findings.

Among protozoan parasites detected in the effluent, *Entamoeba* species showed better elimination than *Cryptosporidium* species. This parasite was detected at Fitch only in *Kiremt* and *Tseday*. This was probably due to low concentration of free ammonia as Fitch digester had the lowest temperature than other digesters and seasons of the study during these periods. Black *et al.* (1982) cited in FAO (1992) mentioned although eggs are resistant, the digestion process destroy cysts of *Entamoeba* species. On the other hand, the existence of *Cryptosporidium* species in the detected seasons may be by virtue of its resistance to the digestion process. The resistance of *Cryptosporidium* oocyst among protozoan to anaerobic digestion was noted by Schonning and Stensron (2004), Oslon (2001) and Chauret *et al.* (1999).

Some experimental work showed the viability of effluent eggs after anaerobic digestion. In this regard, Cram, (1943) as cited in Feachem *et al.* (1978) noted eggs of *Ascaris lumbricoides* and *Taenia saginata* were viable after 20<sup>0</sup>C and 30<sup>0</sup>C digestion with retention days of more than two months. Especially, the viability of *Taenia saginata* even after 200 days of digestion was indicated by Newton *et al.* (1948) in the same book. Similarly, Xun *et al.* (1992) also recommended further treatment of effluent slurry to avoid any remaining eggs as they confirmed that second pollution could occur if this is not achieved.

Therefore the presence of several eggs in the effluent slurry has its own environmental implication. In line with these, all components of the environment, that is, soil, water, plants and animals can be affected as effluent slurries utilized for gardening and discharged to the stream. However, World Health Organization guidelines for the microbiological quality of effluent slurries used for restricted and unrestricted irrigation is equal to or less than one intestinal

nematode egg per liter (WHO, 1989). However, according to this study, there were several eggs of nematode in the effluent slurry unlike the guidelines of World Health Organization.

In another development, according to Feachem *et al.* (1978), protozoa and helminthes do not penetrate healthy undamaged surfaces of vegetables, and die away rapidly on crop surfaces exposed to sunlight. However, pathogens can survive for extended periods inside leafy vegetable or in protected cracks or steam areas. In addition, animals grazing on sewage-irrigated pasture or drinking such sewage can become infected. Unlike bacteria and virus infection, infection with protozoan or helminthes may occur with a small number of ingested organisms (small infective dose).



## 7. CONCLUSIONS AND RECOMMENDATIONS

Although the most resistant egg that was found in the effluent slurry is *Ascaris lumbricoides*, toilet-linked biogas digesters working with in the range of ambient temperature also contain eggs of *Taenia saginata*, *Trichuris trichiura*, *Schistosomia mansoni*, *Fasciola* species and oocysts of *Cryptosporidium* species. In fact, there is difference in time (season) and site (location) with higher number of eggs in a season or site, which has got lower temperature or a combined effect of these two factors since temperature is positively correlated with reduction of intestinal parasites. On the other hand, digesters have better capacity to remove *Entamoeba* species. In general, as a result of this study, effluent slurry of digesters could be a potential source of environmental pollution in general and human infection in particular. Therefore, in order to avoid pollution the following recommendations are forwarded:

1. Digesters should be managed in order to achieve better digestion process. For this achievement, variables, which are important for the process such as total solid, should be managed in a suitable manner so that better digestion process may result in effluent slurry with reduced parasite load.

2. After all management activities, low temperature may not eliminate all parasites. Therefore, mixing urea with effluent slurry may eliminate parasites since the free ammonia concentration level can be enhanced.

3. The other option is composting. This has proved to be an effective process for the destruction of parasites. The mechanisms of destruction are thermal killing, competition for resources and biological antagonism.

4. All digesters (except Fitch) are linked with toilets and the toilets have been giving services for a known number of households and individuals. Thus, periodic mass deworming and treatment of individuals minimize the number of ova of parasites that enters into influent slurry and eventually that release to the environment.

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
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DECLARATION

This thesis is my work, has not been presented for a degree in any university and that all sources of material used for the thesis have been duly acknowledged.

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Date: June 21 2006