

PARASITES IN SOUTHERN SLUDGES AND DISINFECTION
BY STANDARD SLUDGE TREATMENT

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16. ABSTRACT Major objectives were to: (a) assess types and densities of parasites in municipal wastewater sludges in the southern United States, (b) investigate the inactivation of parasites by lime stabilization of sewage sludges seeded with selected intestinal parasites, (c) assess conventional sewage sludge treatment processes from laboratory and field data for the control of parasites. Sludge samples examined in each of the four seasons from 27 municipal wastewater treatment plants indicated the following: (a) viable eggs of <u>Ascaris</u> and <u>Toxocara</u> were observed at least once from every plant, (b) viable eggs of <u>T. vulpis</u> and <u>T. trichiura</u> were observed at least once from 26 and 15 plants, respectively, and (c) viable eggs of at least 10 other helminths and cysts of a few protozoa were observed in fewer numbers and less frequently. Certain drying bed conditions such as previous sludge stabilization, high temperature, and low moisture content appear to inactivate parasite eggs synergistically between 60% to 5% sludge moisture content. Laboratory studies indicate that destruction of resistant parasite eggs is primarily due to temperature and not to a specific digestion process. The application of lime to primary, aerobic digested, and anaerobic digested sludge was found to be effective with >80% reduction of <u>Ascaris</u> viability in 5 days following aerobic digestion at a lime dosage of about 1,000 mg/gram of sludge solids. Laboratory experiments showed that at certain combinations of ultrasonic frequency intensity, and exposure time <u>Toxocara</u> eggs could be destroyed.		
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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

This research grant has measured parasite densities and occurrences in southern sewage sludges. From laboratory and field studies, the following processes have shown some effect in parasite inactivation: drying beds, thermophilic digestion, aerobic digestion, liming and aerobically digested sludges, and ultrasonication of municipal sludges.

Francis T. Mayo, Director
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ABSTRACT

The objectives of this research grant were to (1) assess the presence and densities of resistant stages of parasites in municipal wastewater sludges (sewage) in the southern United States, (2) investigate the inactivation of parasites by lime stabilization of sewage sludges seeded with selected intestinal parasites, (3) measure the mass balance of these parasites through various processes in a municipal wastewater treatment plant, and (4) assess standard sewage sludge treatment processes from laboratory and field data for the control of parasites. Sludge samples collected during each of the four seasons from 27 municipal wastewater plants located in Alabama, Florida, Mississippi, Louisiana and Texas were examined for the presence and densities of resistant stages of human and animal parasites. Viable eggs of Ascaris and Toxocara were recovered at least once from every plant and viable eggs of T. vulpis and T. trichiura were recovered at least once from 26 and 15 plants, respectively. Viable eggs of at least 10 other helminths and cysts of a few protozoa were also found in fewer numbers and less frequently. Certain drying bed conditions such as previous sludge stabilization, high temperature and low moisture content appear to inactivate parasites eggs synergistically between 60% to 5% sludge moisture content. Results of the mass balance study indicated that the clarifier processes tended to concentrate and equalize parasite concentrations; the clarifier overflow rate affected removal efficiency of parasites. Laboratory studies verified results of previous investigations indicating that destruction of resistant parasite eggs is primarily due to temperature and not to a specific digestion process. The application of lime to primary, aerobic-digested, and anaerobic-digested sludge was found to be effective with >80% reduction of Ascaris viability in five days following aerobic digestion at a lime dosage of about 1,000 mg/gram of sludge solids. Laboratory experiments also showed that at certain combinations of ultrasonic frequency intensity, and exposure time Toxocara eggs could be destroyed, but that the same ultrasonic conditions did not affect Ascaris eggs. This study also demonstrated that eggs taken from the uteri of live Ascaris females are not suitable for use as an indicator parasite in laboratory inactivation studies.

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CONTENTS

Foreward	iii
Abstract	iv
Figures	vi
Tables	viii
Acknowledgement	x
1. Introduction	1
2. Conclusions	3
3. Recommendations	5
4. General Background	6
5. Methodology	22
6. Results and Discussion	36
Field Studies	36
Laboratory Studies	70
References	92
Appendices	
A. Procedure for Parasitologic Examination of Waste Sludges . . .	99
B. Results of Field Investigation	105
C. Results of the Drying Bed Study	168
D. Calculations on the Effectiveness of Wastewater Treatment Process on Parasite Reductions in the Field	171
E. Results of Laboratory Studies Containing Raw and Analyzed Data	179

FIGURES

<u>Number</u>	<u>Page</u>
1 Statistical analysis of drying bed sample	25
2 Geographic regions in which municipal waste treatment plants were studied	45
3 Average percent reduction of viable parasite eggs versus unit process	51
4 Percent reduction of viable parasite eggs in drying bed dewatered sludges following anaerobic or aerobic stabilization as com- pared to densities in raw sludge versus seasons and yearly averages.	53
5 Percent reduction of viable parasite eggs in drying bed de- watered sludges following aerobic stabilization as compared to densities in raw sludges versus seasons and yearly averages . .	54
6 Percent reduction of viable parasite eggs in drying bed de- watered sludges following aerobic stabilization as compared to densities in raw sludges versus seasons and yearly averages . .	55
7 Percent reduction of viable <u>Toxocara</u> eggs in drying bed de- watered sludges following anaerobic, aerobic or both stabili- zation processes as compared to parasites in raw sludge versus season and yearly average	57
8 Percent reduction of viable <u>Ascaris</u> eggs in drying bed de- watered sludges following anaerobic, aerobic or both stabili- zation processes as compared to parasites in raw sludge versus season and yearly average	58
9 Percent reduction of viable <u>T. trichiura</u> eggs in drying bed de- watered sludges following anaerobic, aerobic or both stabili- zation processes as compared to parasites in raw sludge versus season and yearly average	59
10 Percent reduction of viable <u>T. vulpis</u> eggs in drying bed dewatered sludges following anaerobic, aerobic or both stabilization pro- cesses as compared to parasites in raw sludge versus season and yearly average.	60

<u>Number</u>	<u>Page</u>
11 Plot of log of viable <u>Ascaris</u> eggs in drying beds versus log of viable <u>Ascaris</u> eggs in raw sludge with respect of moisture content	61
12 Plot of log of total <u>Ascaris</u> eggs in drying beds versus log of total <u>Ascaris</u> eggs in raw sludge with respect of moisture content	62
13 Percentage of viable <u>Toxocara</u> and <u>Ascaris</u> eggs versus percentage of moisture content	64
14 Percentage of viable <u>Ascaris</u> eggs versus percentage moisture content for each season in drying beds	67
15 Percentage of viable <u>Toxocara</u> eggs versus percentage moisture content for each season in drying beds	68
16 Schematic of domestic waste treatment facility investigated for mass balance	70
17 Percent removal of viable <u>Ascaris</u> eggs with respect to overflow rate by secondary clarifier	73
18 Effects of aerobic digestion on <u>Ascaris</u> eggs (from the uteri of worms)	75
19 Effects of aerobic digestion on <u>Toxocara</u> eggs (from dog feces) .	76
20 Effects of aerobic digestion on <u>Ascaris</u> eggs (from the small intestinal contents of swine)	77
21 Effects of anaerobic digestion at 35°C, 45°C and 55°C on <u>Ascaris</u> eggs (from the small intestinal contents of swine) and <u>Toxocara</u> eggs (from the feces of dogs)	79
22 Effect of lime stabilization on <u>Ascaris</u> eggs (from the small intestines). Raw primary sludge was limed, and then maintained under aerobic conditions at ambient temperature.	82
23 Effects of lime stabilization on the viability of <u>Ascaris</u> eggs in aerobically-digested sludge at 28°C under aerobic and anaerobic environments at ambient temperature	84
24 Effects of lime stabilization on the viability of <u>Ascaris</u> eggs in aerobically-digested sludge at 28°C under aerobic and anaerobic environments at ambient temperature	86
25 Effects of ammonia on the viability of <u>Ascaris</u> eggs in lime-stabilized aerobically-digested sludge under anaerobic conditions	87

TABLES

<u>Number</u>	<u>Page</u>
1 Parasites of medical importance that might be present in sewage sludges in the United States and whose resistant stages are likely to remain viable while passing through conventional sludge treatment processes	8
2 Parasites of medical importance that might be present in raw sewage sludges in the United States, but whose free-living stages are unlikely to remain viable while passing through conventional sludge treatment processes	9
3 Effects of waste treatment processes on parasite eggs and cysts . .	15
4 Methodology for selecting waste treatment plants	22
5 Wastewater treatment and sludge treatment processes investigated . .	22
6 Statistical analysis of drying bed sample.	26
7 Parasites found in sludge samples from 27 municipal plants in southern United States	36
8 Number of municipal plants in which eggs of <u>Ascaris</u> , <u>Toxocara</u> , <u>Trichuris trichiura</u> and <u>Trichuris vulpis</u> were found.	38
9 Miscellaneous parasites found in sludges from 27 municipal treatment plants sampled	38
10 Average number of eggs of <u>Ascaris</u> , <u>Toxocara</u> , <u>Trichuris trichiura</u> and <u>Trichuris vulpis</u> eggs found in plants in each geographic region in each season	40
11 Average number of viable eggs of <u>Ascaris</u> , <u>Toxocara</u> , <u>Trichuris trichiura</u> and <u>Trichuris vulpis</u> found in raw sludges in plants in each geographic region in each season	41
12 Average number of viable eggs of <u>Ascaris</u> , <u>Toxocara</u> , <u>Trichuris trichiura</u> , and <u>Trichuris vulpis</u> found in treated sludges in plants in each geographic region in each season	42
13 Parasite concentrations in primary and secondary sludge as compared to treated sludge.	46

<u>Number</u>		<u>Page</u>
14	Influence of abattoir wastes on parasite concentrations in primary and secondary sludges	48
15	Average percent reduction of viable parasite eggs by unit processes	50
16	Percent reduction of viable parasite eggs by total sludge treatment processes	52
17	Statistical data on regression analysis for log-log plots on viable and total <u>Ascaris</u> eggs comparing parasite densities from the raw sludge to final drying bed sludges	63
18	Drying bed samples in which no viable <u>Ascaris</u> or <u>Toxocara</u> eggs were recovered in the different seasons	65
19	Waste characteristics of influent raw wastewater for plant receiving abattoir waste	71
20	Effectiveness of secondary clarification to remove <u>Ascaris</u> eggs from wastewater for mass balance study	72
21	Batch aerobic digestion of raw primary sludge spiked with <u>Ascaris</u> eggs (at 28°C).	78
22	Influence of anaerobic digestion at 35°, 45° and 55°C on the viability of <u>Ascaris</u> and <u>Toxocara</u> eggs in raw secondary sludge over a fifteen-day period	78
23	Effects of lime stabilization on <u>Ascaris</u> and <u>Toxocara</u> eggs in primary sludge under aerobic conditions	81
24	Effects of lime stabilization on <u>Ascaris</u> eggs added to sludges and then maintained at ambient temperatures under either anaerobic or aerobic conditions after lime addition	83
25	Effect of ammonia on <u>Ascaris</u> eggs and <u>Toxocara</u> eggs in 28 and 35°C lime-stabilized aerobically-digested sludge per gram suspended solids under anaerobic conditions and in 35°C anaerobically-digested sludge at ambient temperatures	88
26	The effect of ultrasonics on <u>Ascaris</u> and <u>Toxocara</u> eggs as evaluated by direct observation	90
27	The effect of ultrasonics on the viability of <u>Ascaris</u> and <u>Toxocara</u> eggs as determined by culturing techniques	91

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SECTION 1

INTRODUCTION

This investigation was developed as a result of collaboration between Tulane University and the U.S. Environmental Protection Agency. The objectives of this study were to (1) assess the presence and levels of resistant stages of parasites in municipal wastewater (sewage) sludges in the southern United States, (2) investigate parasite inactivation by lime stabilization of sludges seeded with selected intestinal parasites, (3) evaluate various wastewater sludge treatment techniques for the control of parasites in municipal sludges and (4) conduct a mass balance analysis of parasites through various stages of a municipal waste treatment system.

In the United States, land disposal of sewage sludge has been practiced with care because of the controversies over possible health and nuisance problems arising from such practice. Nevertheless, land disposal is relatively common and does constitute a major disposal practice in some states; for example, Ohio disposes of approximately 60% of its municipal sludges by land application (1).

Questions concerning potential disease transmission have prevented greater application of sewage sludges on land. A number of studies have shown that both viruses and bacteria are associated with the sludge suspended solids and are thereby concentrated in the sewage sludge (2,3).

Until recently, evidence pointed to the effective destruction of pathogens by anaerobic digestion. Unfortunately, research has now shown that this is not entirely true (3-5). Even though most microorganisms are thought to be destroyed during anaerobic sludge digestion, a number of microbes have been shown to survive this process--e.g., bacteria (Salmonella typhosa), parasites (various helminth eggs), and viruses (polio virus) (3-6). The survival of these microorganisms in stabilized sludges has caused many health authorities to remain skeptical about the practice of land disposal for sewage sludges because of concern that viable pathogens may be dispersed in the environment. Many pathogens are known to survive in soil for days, months or possibly years (e.g., the tubercle bacilli and the eggs of the nematode Ascaris).

Even though anaerobic digestion of sludges cannot be relied upon to effectively destroy every pathogen, there are a number of processes by which known pathogens can be destroyed. Pasteurization of sludges is practiced in some areas of Europe where it is reported to inactivate all pathogens when temperatures of 70°C are attained for at least 30 minutes. The use of the

pasteurization process, however, requires considerable quantities of heat, and may not be always feasible since it is energy intensive. Other recent investigations suggest that pathogens in sewage sludges can be completely destroyed by composting prior to use. However, composting, like pasteurization, may be too costly at the present time. Massive doses of chlorine will also destroy most pathogenic microbes in sewage; however, the effects of the chlorinated residues on the environment are unknown.

A possible alternative, conducive to utilization of the waste, lies in lime stabilization of sludges. The liming process does not have high energy requirements and does not produce potentially hazardous residues. When lime is applied to sewage (sludge), the pH can be increased to 12 which inhibits or substantially reduces the activity of certain microbes including those of fecal origin (6). Also by raising the pH to high levels, odorous by-products (hydrogen sulfide and various mercaptans) are controlled by reducing these by-products and keeping them in a charged state (7). The literature on the fate of pathogens in lime-stabilized sludges indicates a reduction in Salmonella typhosa, Escherichia coli, Salmonella spp. and Pseudomonas aeruginosa along with nuisance odors at pHs around 12 (8-13). At high pH levels, solubilization of most heavy metals is also reduced (especially metals of a cationic nature). Collectively, these factors increase the acceptability of land disposition of lime-stabilized sludges.

In investigations on pathogenic organisms in sludge, parasites have received the least attention. Given the current state of knowledge, there is a need for further assessment of the health problems related to the presence of parasites in sludges, as well as the examination of various sludge treatment methods on parasite survival. A few studies were published in the 1940's and 1950's but between 1960 and 1975 little was reported in the United States literature on parasite transformation through sewage sludges (14-18). It was the purpose of this study to attempt to fill some of the gaps in our knowledge on the occurrence of human or animal parasites in municipal sludges in the United States and to investigate select methods for inactivating those parasites.

SECTION 2

CONCLUSIONS

Generally, the influence of sludge handling, treatment and disposal along with the effect of wastewater treatment processes on parasite survival is very complex and influenced by many parameters. Such factors include the type of parasite, temperature, moisture content, etc. Field studies in this investigation of southern municipal sewage sludges showed that:

- 1) Most raw samples contained viable parasite eggs and cysts.
- 2) Eighteen species of parasites (eggs or cysts) were observed in both stabilized and raw sludges.
- 3) Eggs of the most prevalent parasites were present in relatively high numbers (an average of 1,000 to 10,000 eggs per kilogram of dry sludge, depending upon the parasite).
- 4) The types of parasite eggs and their densities were found to vary with population served, type of industrial contribution, season of the year, and geographical region.
- 5) Abattoir and packing plant wastes may significantly influence the types and densities of parasite eggs found in domestic waste sludges.
- 6) Conventional sludge stabilization processes alone were not very effective on inactivating parasites in field samples. Depending upon the parasite, however, a particular stabilization process coupled with sludge drying bed, may inactivate parasite eggs.
- 7) Sludge thickening and dewatering processes (vacuum filtration and centrifugation) tended only to concentrate parasites in the sludge.
- 8) Certain sludge drying bed conditions such as previous sludge stabilization, high temperature and low moisture content appear to inactivate parasite eggs synergistically between 60% to 5% moisture content.
- 9) From the parasite mass balance study it was observed that secondary clarifiers tend to concentrate parasites in the return sludges. Increased overflow rates through clarifiers appear to increase the densities of parasites in clarifier effluents.

The results of laboratory studies on parasite inactivation by aerobic digestion, anaerobic digestion, lime stabilization, ammonia treatment, and ultrasonication indicated the following:

- 1) Aerobic digestion inactivated parasite eggs at temperatures of 55°C or greater within two hours, and at 45°C within two days.
- 2) Anaerobic digestion inactivated Ascaris and Toxocara eggs at temperatures greater than 45°C, but only retarded egg development at temperatures less than 45°C.
- 3) Lime stabilization produced noticeable reduction in the viability of Ascaris eggs. Under aerobic conditions at ambient temperatures with dosages of 1,000 mg or greater of lime per gram of dry sludge solids, the viability of Ascaris eggs was observed to be reduced over 80% within five days in primary, 28°C aerobically digested or 35°C aerobically digested sludges. Under anaerobic conditions at ambient temperatures with lime dosages of 100 mg of lime per gram of dry sludge solids, a 100% reduction of viable Ascaris eggs was noted within 20 days in 28°C aerobically digested sludge.
- 4) The results of the ammonification studies were inconclusive. In aerobically digested sludges, 95% of the viable Ascaris egg densities were reduced within 5 days, even in the control (no ammonia added). In anaerobically digested sludges no reductions in Ascaris viability were observed at any dosage of ammonia up to 5000 milligrams of ammonia sulfate per gram of sludge suspended solids.
- 5) Ultrasonication was effective in destroying Toxocara eggs at 49 kilohertz (kHz) and 26 watts within a 9 minute exposure and at 64 kHz and 74 watts within a 6 minute exposure; but ultrasonication was not effective in destroying Ascaris eggs under these same conditions.

SECTION 3

RECOMMENDATIONS

The results of this one and one-half year study on the incidence and persistence of parasites in sewage sludge indicate that additional information can be obtained by further research. The specific areas recommended for additional research on the fate of parasites in wastewater sludges are as follows:

- 1) The investigation of selected municipal sludges in the northern portion of the United States for the presence and density levels of resistant stages of parasites.
- 2) A comparison of the sludge data from the northern portion of the United States to the data collected in the southern part of the United States.
- 3) The development of a standard analytical method for parasitologic examination of sewage sludges.
- 4) An evaluation of promising techniques for their effectiveness in inactivating and/or destroying parasites in sewage sludges.

Recent literature studies support potential viable alternatives for parasite decontamination of sludges. Four such alternatives are:

- 1) ultrasonication
- 2) lime-stabilization with ammonia additions
- 3) drying beds
- 4) aerobic digestion with the aid of surface active agents.

The choice of one or more of these four alternatives would be based on economics, low maintenance and energy requirements, effectiveness of treatment, and ease of incorporation into current sludge stabilization techniques.

Finally, the potential health risks due to the presence of infectious agents in sludge should be determined. Currently, risk assessment is being studied by epidemiological researchers; but, the quantitative risk assessment due to parasites (i.e., Ascaris, etc.) in sludge has yet to be determined.

SECTION 4

GENERAL BACKGROUND

DOMESTIC WASTEWATER SLUDGE REUSE

According to the Federal Water Pollution Control Act Amendments of 1972 (PL 92-500), land application of domestic sewage sludge must be considered as a disposal alternative to land-filling, incineration, and ocean dumping. The resources in these sludges, however, can be recycled by land application making this technique a viable alternative to the above mentioned disposal methods. Municipal wastewater sludges have been used as soil additives and/or fertilizers and on truck gardens, flower beds, private lawns, worm farms, school grounds, public parks, football fields, plant nurseries, citrus groves, golf courses, and roadsides. Sewage sludges are also added to bagged products or used as additives to various fertilizer products and offered for sale.

In the United States, there has been minimal concern over the presence of parasites in domestic waste. However, in many countries which utilize sludge for land application, parasites in sewage have posed serious health problems. During the past five years, research concerning public health problems of municipal sludges has been conducted. These investigations generally have been concerned with pathogenic microbes, enteric viruses, heavy metals, and toxic chemicals. There is a scarcity of information concerning parasites in municipal sludges and treatment thereof.

PARASITES IN SLUDGE

Since the early part of this century, public health workers in the United States have been concerned about the presence of resistant stages of human and animal parasites in domestic sewage effluents and sludges as a possible source of disease. However, until recent years there were relatively few published reports of studies concerning this problem. According to Bond, 1958 (17), Dr. Homer Venters, a public health laboratory worker, found, in 1918, eggs of Ascaris in 44 percent of 200 samples of sludge from Imhoff tanks in Tampa, Florida. In 1942, Wright et al. (14) examined sludge samples from 16 municipal treatment plants and two national parks in California, from one national park in Arizona, and from 17 U.S. Army camps in eight southern states. They found parasite eggs (Ascaris, Trichuris trichiura, and Hymenolepis sp.) in sludges from 7 of the 17 Army camps, but found only one positive parasite sludge sample from the municipal wastewater treatment plants and national parks (one egg of Hymenolepis sp. was found in a sample from Whittier, California).

In 1954, Wang and Dunlop (18), studied a sewage plant in Denver,

Colorado, and the adjacent South Platte River for the presence of parasites. Ascaris eggs were found in all 11 samples of each raw sewage and primary settled sewage; in 1 of 11 samples of the river above the plant, in 9 of 11 samples of the sewage plant effluent, and in 10 of 11 samples in the river below the sewage plant. They also reported finding eggs of Trichostrongylus, Taenia, and Trichuris in low numbers from the settled sewage or effluent. Cysts of Entamoeba coli, a non-pathogenic amoeba, were found in samples of raw sewage, plant effluent, and in the river above and below the plant.

Bond (17) examined raw, digested and drying bed sludge samples from a municipal sewage treatment plant in Tampa, Florida, during the period from November, 1955 to January 1956. Ascaris eggs were observed in some samples of each type of sludge.

Lepak, in 1961 (19), studied a culinary water supply system in the Salt Lake City area for possible contamination with animal parasites. He recovered Ascaris eggs from untreated water from a reservoir at the point of entrance to the Salt Lake aqueduct. He also examined raw, primary and secondary effluent from the Heber City sewage plant and recovered Entamoeba coli cysts from the effluent.

In recent years, changes in regulations concerning the treatment of municipal wastewaters have resulted in a magnitudinal increase in the production of domestic sewage sludges. The problems related to the disposal of these sludges have increased accordingly. Among the alternative methods of sludge disposal, those that "recycle" the sludge through land applications have attracted increasing interest. However, the health risks to humans and animals resulting from the disposal of sludges by land application are not completely understood. A number of studies have been initiated in the past few years to determine the potential risks due to the presence of infectious agents in sludges (i.e., viruses, bacteria, parasites and fungi).

Fox and Fitzgerald (20,21) conducted a comprehensive study of parasites in raw sewage, anaerobically digested sludges and effluents from the Metropolitan Sanitary District of Greater Chicago. They reported finding eggs of Ascaris, Toxocara, Toxascaris, Trichuris, Taenia, Hymenolepis and Enterobius, and cysts of Elmeria, Isospora and Entamoeba coli. M.D. Little (unpublished data, 1976) examined raw, aerobically and anaerobically digested, and lime-stabilized domestic sludge samples from Lebanon, Ohio, and found eggs of Ascaris, Toxocara, Trichuris trichiura, Trichuris vulpis and Enterobius vermicularis. Hays (22) examined raw and digested sludge samples from four sewage plants in Allegheny Co., Pennsylvania, and found eggs of Ascaris, Trichuris trichiura, Capillaria hepatica, Enterobius vermicularis, Hymenolepis nana and H. diminuta.

In a study supported by the Environmental Protection Agency, the East Bay Municipal Utility District of Oakland, California, and the Sacramento Regional Sanitation District, Theis et al., in 1978 (23), examined municipal sludges (raw, digested, and composted) from 12 areas of the United States. They reported Ascaris and Toxocara eggs in sludges (raw, digested or composted) from Oakland, Los Angeles and Sacramento, California; Macon, Georgia,

Springfield; Missouri, Hopkinsville, Kentucky; and Frankfort, Indiana. Trichuris, Toxascaris and Hymenolepis diminuta eggs were found in raw digested or composted sludges from Los Angeles and Oakland, and Taenia and H. nana eggs were found in sludges from Los Angeles. Helminth eggs were not found in samples from Las Virgenes, California; Kendalville, Indiana; Columbus, Indiana; Wilmington, Ohio, or Chippewa Falls, Wisconsin.

Jackson et al. (24) reported that in a 1975 study supported by the United States Food and Drug Administration, Chaney and Burge found Ascaris eggs in liquid sludge samples from 16 municipalities in the United States. Viable Ascaris eggs were present in samples from 13 of the 16 cities.

The resistant free-living stages of the intestinal parasites of man, domestic animals, pets, and wild animals (i.e., the eggs and larvae of helminths and the cysts of protozoa) are the stages of parasites that are most likely to enter sewage systems. Several authors have provided lists of parasites that might be encountered in municipal sewage sludges (22,25); however, in these lists the relative importance of different parasites in sludges was not evaluated.

POSSIBLE PROBLEM PARASITES

In Table 1 are listed the parasites most likely to be present in the sludges of this country which may produce disease in man, and which have resistant stages that are likely to survive sludge treatment processes. Table 2 lists parasites that might be present in sludges and can infect in man but which have free-living stages (eggs, larvae, or cysts) that are unlikely to remain viable while passing through sludge treatment processes.

Ascaris lumbricoides is perhaps the most important and the most commonly found parasite in stabilized and raw sludges (26). The egg of A. lumbricoides is resistant to a wide range of physical-chemical environments which enables it to survive conventional sludge treatment techniques. The eggs of Ascaris are not in an infective stage to man when they enter the sewage treatment system, but may develop to infectivity in approximately three weeks under proper conditions (sufficient moisture and oxygen, and optimum temperatures).

TABLE 1. PARASITES OF MEDICAL IMPORTANCE THAT MIGHT BE PRESENT IN SEWAGE SLUDGES IN THE UNITED STATES AND WHOSE RESISTANT STAGES ARE LIKELY TO REMAIN VIABLE WHILE PASSING THROUGH CONVENTIONAL SLUDGE TREATMENT PROCESSES

Parasite	Stage	Definitive Host
<u>Ascaris lumbricoides</u>	Egg	Man
<u>Ascaris suum</u>	Egg	Pig
<u>Trichuris trichiura</u>	Egg	Man

(continued)

TABLE 1. (continued)

Parasite	Stage	Definitive Host
<u>Trichuris suis</u> ¹	Egg	Pig
<u>Trichuris vulpis</u> ¹	Egg	Dog
<u>Toxocara canis</u>	Egg	Dog
<u>Toxocara cati</u>	Egg	Cat
<u>Taenia saginata</u> ²	Egg	Man
<u>Taenia solium</u>	Egg	Man
<u>Echinococcus granulosus</u>	Egg	Dog
<u>Echinococcus multilocularis</u>	Egg	Dog
<u>Toxoplasma gondii</u>	Oocysts	Cat

¹ Medical importance questionable.

² Egg not infective for man.

TABLE 2. PARASITES OF MEDICAL IMPORTANCE THAT MIGHT BE PRESENT IN RAW SEWAGE SLUDGES IN THE UNITED STATES, BUT WHOSE FREE-LIVING STAGES ARE UNLIKELY TO REMAIN VIABLE WHILE PASSING THROUGH CONVENTIONAL SLUDGE TREATMENT PROCESSES

Helminths	Stage	Definitive Host
<u>Enterobius vermicularis</u>	Egg	Man
<u>Necator americanus</u>	Egg (larvae) ¹	Man
<u>Ancylostoma braziliense</u>	Egg (larvae) ¹	Dog, Cat
<u>Ancylostoma caninum</u>	Egg (larvae) ¹	Dog
<u>Strongyloides stercoralis</u>	Larvae	Man (dog?)
<u>Hymenolepis nana</u>	Egg	Man (rodents?)

(continued)

TABLE 2. (continued)

Protozoa	Stage	Definitive Host
<u>Entamoeba histolytica</u>	Cysts	Man
<u>Giardia lamblia</u>	Cysts	Man (animals?)
<u>Balantidium coli</u>	Cysts	Pig, man

1 Under suitable conditions larvae hatch from eggs in 24-48 hrs and larvae could be present in sewage.

Humans acquire infections by swallowing infective eggs. Disease due to A. lumbricoides is usually related to the level of infection, i.e., the number of worms acquired. In heavy infections, the absorption of nutrients by the intestine is usually impaired. Also, large numbers of worms can cause a blockage of the intestine. Ascaris pneumonitis (Loeffler's syndrome) is common in areas where transmission of the parasite occurs seasonally, and this condition can result from the ingestion of only a small number of eggs. Even in light infections, disease is possible from the migration of one or more adult worms to an extraintestinal location, such as the bile duct or liver.

Ascaris suum is a parasite of pigs and is found in many parts of this country. The eggs of A. suum are indistinguishable from those of A. lumbricoides and are apparently just as resistant to environmental conditions. Human infections due to A. suum have been reported and in three cases serious pulmonary complications resulted from the accidental ingestion of large numbers of eggs (27).

Trichuris trichiura is the common whipworm of humans. The adult worms live in the large intestine with the anterior portion of their body threaded superficially through the mucosa. Eggs are passed in the feces of the infected person and under suitable conditions in the soil will develop to the infective stage in about four weeks. Infections result from the ingestion of infective eggs from the soil. Disease is related to the number of worms present in the body, and in heavy infections, chronic diarrhea or dysentery may be present.

Trichuris suis is the whipworm of pigs and its life cycle is similar to that of T. trichiura. Naturally acquired infections in humans have not been confirmed but one successful experimental infection in a 23-year old male has been reported (28). It is possible that a heavy infection in a human could produce colitis.

Trichuris vulpis is the whipworm of dogs. Its eggs are much larger than

those of T. trichiura and are sufficiently distinctive that they can be readily identified. Its life cycle is similar to that of T. trichiura. Several human cases of infection with T. vulpis have been reported (29) but were apparently all without symptoms.

Toxocara canis is the common roundworm of dogs and is found in dogs in most parts of this country. The life cycle of this parasite is complex with transmission of infection to dogs occurring in a number of ways including: 1) ingestion of infective eggs from soil, 2) ingestion of a paratenic host, such as a rodent, which has infective larvae in its tissues, or 3) trans-uterine migration of larvae from the tissues of the bitch to the tissues of the fetuses. Eggs passed in the feces of the dog can develop to the infective stage in two to three weeks under suitable conditions. When infective eggs are accidentally ingested by man (mainly children), larvae migrate through the tissues producing a condition known as visceral larva migrans (30). In numerous cases, the larvae have migrated into the eye causing a lesion similar to that of retinoblastoma that lead to the loss of the eye (31).

Toxocara cati is the common roundworm of cats. Like T. canis it has a complex life cycle with cats acquiring infections by: 1) ingesting infective eggs from the soil, 2) eating infected paratenic hosts such as rodents, or 3) the transmission of larvae through the milk of the mother cat to the newborn kitten (i.e., by transmammary transmission) (32). A few human cases of visceral larva migrans due to T. cati have been reported (33,34).

Taenia saginata is a large tapeworm of humans that is only occasionally found in this country. However, human infections are relatively common in Mexico, Central and South America, and in other areas of the world where beef is eaten. T. saginata requires an intermediate host which is bovine. Humans become infected by eating raw or inadequately cooked beef containing the larval stage of the tapeworm, the cysticercus. The eggs of T. saginata are not infective to man. Cattle become infected by grazing on pastures where eggs have been disseminated by proglottids (segments of the worm) passed in human feces.

Taenia solium is also a large tapeworm which in the adult stage occurs only in man. Locally acquired cases rarely, if ever, occur in the United States, but infections are occasionally seen in persons who have acquired the infections elsewhere, usually in Mexico or Central America. The pig is the usual intermediate host of this parasite and human infections result from eating pork that has been inadequately cooked. Pigs become infected by ingesting eggs that have been dispersed by proglottids passed in the feces of infected humans. This tapeworm is potentially more dangerous to humans than is T. saginata since the eggs of T. solium are infective to man. When ingested by man, larvae hatch from the eggs, migrate into the tissues and develop to the cysticercus stage. The resulting infection, called cysticercosis, is often serious, especially when the cysticerci are located in the brain or the eye.

Echinococcus granulosus is a small tapeworm that utilizes canines as the

definitive host. Its larval stage, the hydatid, may occur in a wide variety of intermediate hosts: sheep, goats, pigs, deer, etc. In man, the larval stage causes hydatid disease. In recent years, the parasite has been found to be primarily restricted to the western states of this country, i.e., California, Utah, Arizona, New Mexico, Nevada, and Idaho. In the past 15 years, autochthonous human infections have been found in California, Utah, Arizona, and New Mexico (35). Humans become infected by accidentally ingesting eggs that have been passed in feces of infected dogs.

Echinococcus multilocularis is a tapeworm parasite of wild canines that causes alveolar hydatid disease in humans. In North America, foxes are the natural definitive hosts with wild rodents serving as intermediate hosts. The parasite is present in sylvatic hosts in North Dakota, South Dakota, Iowa, Minnesota, Montana, Wyoming, Alaska and Canada. Humans can become infected by ingesting the eggs passed in the feces of definitive hosts, yet only one human case has been reported from the United States, i.e., a woman in Minnesota (36). Domestic dogs and cats can become infected with the adult stage if they prey on infected rodents.

Toxoplasma gondii is a protozoan that infects a wide variety of animals. Domestic cats and related felines serve as the natural hosts. The oocyst, which passes in the feces of infected cats, is relatively resistant to a wide range of environmental conditions. Oocysts have been reported to survive in the soil for at least 18 months (37). Humans become infected by either ingesting the oocyst, by eating raw or undercooked meat containing intracellular forms of the parasite, or by congenital transmission. Toxoplasma infections in humans (toxoplasmosis) are common in the United States but serious disease is primarily seen in infants who have congenital infections and in immunologically deficient persons. Rarely is acute toxoplasmosis seen in previously healthy individuals.

FACTORS INFLUENCING THE FATE OF PARASITE EGGS AND CYSTS

There are many abiotic and biotic variables which may affect the resistant stages of parasites. In general, these parameters can be divided into three categories: chemical (ammonia, hydrogen cyanide, etc.), physical (temperature, irradiation, moisture content, etc.) and biological (fungi, protozoa, and invertebrates).

The effect of various chemicals on the eggs of Ascaris has been studied by numerous workers. These studies which demonstrate the remarkable resistance of Ascaris eggs to chemicals have been reviewed by Fairbairn (38) and Morishita (26). It has been reported that Ascaris eggs will develop to the infective stage in a wide range of relatively toxic solutions such as 14% hydrochloric acid, 9% sulfuric acid, 8% acetic acid, 0.4% nitric acid, 0.3% carbonic acid, 0.5% sodium hydroxide, 1% mercuric chloride, and 4% formaldehyde. The resistance of these eggs to toxic substances is mainly due to the relatively impermeable inner membrane of the shell which is lipoid in nature. This lipoid membrane is, however, altered by many organic solvents, including chloroform, ethyl ether, alcohols, phenols, and cresols. Some surface active agents have also been noted to damage this membrane. It is permeable to respiratory and certain noxious gases, e.g., methyl bromide,

hydrogen cyanide, hydrozoic acid, ammonia, and carbon monoxide, which can kill the developing embryo (38,39). However, the charged forms of these gases will not penetrate the lipoid membrane (38). Ozone and chlorine have been found to be capable of killing Schistosoma mansoni eggs when present at levels of 4.0 mg/l and 40 mg/l, respectively (40). However, ozone appears to have no effect on the eggs of Ascaris or Hymenolepis (41), and routine doses of chlorine in wastewater have no effect on parasite eggs (42).

The physical factors which appear to influence the viability of Ascaris eggs are temperature, irradiation, moisture content, and sonication. Generally, Ascaris eggs are resistant to high temperatures, but it has been reported that heating to 55°C for one hour or 45°C for 20 days will completely destroy the eggs (24,43,44). Also, eggs placed in water and frozen at temperatures of -21° to -27°C were dead by the 20th day of freezing (45). Moisture content of the surrounding medium also affects the viability of parasite eggs. Nolf (46) found that there was complete inhibition of Ascaris and Trichuris trichiura eggs after 4 days at 25° to 30°C when the relative humidity was 40 to 50% in soils. Cram, however, found that viable eggs could survive in stabilized sludge with a moisture content as low as 4% (15). Irradiation has been shown to affect the viability of Ascaris eggs. Alexandre et al. (47) reported that Parascaris eggs which still have their outer coat were not affected by even 1800 krads of radiation, but were susceptible to 400 krads if the outer coat was not present. Brandon (44) reported that thermoradiation may be used to disinfect sludge. However, in this study the eggs that were tested had previously had the outer layer of the shell removed. The effects of sonication and ozonation on the eggs of Ascaris suum, Nippostrongylus brasiliensis, Schistosoma mansoni, and Hymenolepis diminuta have been evaluated by Burleson and Pollard (41). At a sonication level of 800 kHerz and 100 watts, N. brasiliensis eggs were destroyed after 15 seconds. The eggs of the other three species proved to be more resistant and ozonation was required to supplement sonication. S. mansoni eggs were killed after 3½ minutes. There were no effects on Ascaris and Hymenolepis eggs after 10 minutes of sonication.

The biological factors which have been shown to affect parasite eggs include fungi and various invertebrates. One fungus which has been shown to penetrate and destroy Ascaris eggs is Cylindrocarpon radicola (48). Invertebrates, particularly insects and gastropods, can also destroy helminth eggs by mechanically breaking the eggs and ingesting them (49,50).

PARASITE TRANSMISSION THROUGH SLUDGES

The use of raw sewage or "night soil" to fertilize vegetable crops is a common practice in many countries and is one of the major factors influencing the transmission of several of the common intestinal parasites of man, especially Ascaris and Trichuris. In these countries, adults mainly acquire infections by eating contaminated vegetables (51). However, children mainly obtain infections by ingesting eggs directly from the soil; i.e., by eating contaminated soil (pica) or by placing in their mouths, fingers, toys, etc. which have been contaminated with soil containing infective stages of parasites.

While raw sewage is seldom, if ever, used in this country for the purpose of fertilizing food crops, the accidental contamination of drinking water with raw sewage has led to occasional outbreaks of parasite infections. Examples include the recent outbreaks of Giardia lamblia infections in Aspen, Colorado, and in Rome, New York (52,53). On the other hand, treated sewage and sludges have been applied to the land in many parts of this country for many years without apparent parasite transmission. The health risk of parasite infection as a result of the land application of sludges needs additional study.

While it seems certain that viable stages of parasites have been present in some of the treated sewage and sludges that have been applied to land there appears to be no report of outbreaks of parasite infections in humans. However, due to the difficulty of relating the parasite infection to a common source, sporadic cases of parasitism acquired from disposal of municipal sludges may go unreported.

Clark et al. (54) recently reviewed the literature pertaining to parasite infections associated with workers in the wastewater industry throughout various parts of the world. The investigators found that these workers, as compared to other population groups, had only a slight increase in parasite infections such as Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale and Entamoeba histolytica.

Most of the literature pertaining to parasitic infections of domestic animals resulting from the use of wastewater effluents or sludges on pastures or animal food crops are concerned with the transmission of Taenia saginata to cattle. In 1937, it was found that 46% of the cattle that had grazed six months on farms irrigated with untreated sewage from Melbourne, Australia, were infected with the cysticerci of Taenia saginata (55). A recent study of cattle reared on pastures irrigated with sewage effluent in the Melbourne area revealed that 52% of the 10-to 11-month-old cattle and 8% of all cattle examined harbored cysticerci of T. saginata (56). Interestingly, there appeared to be a higher incidence of infection due to primary wastewater effluent applications than sludge applications. In 1935, Roberts (57) detected T. saginata infections in cattle on a farm near Tucson, Arizona, which had probably acquired their infections by drinking primary sewage effluent. Northington (58) mentioned a problem of T. saginata infections in cattle near Munich after World War II that had been raised on pastures to which primary sewage effluents and raw primary sludges had been applied. Also, it was recently reported that cysticercosis due to T. saginata was detected in cattle raised on pastures in southern Virginia that had received applications of domestic sludges (59). However, the possibility that some of the sludges applied to these pastures were untreated has been raised.

EFFECTS OF WASTEWATER TREATMENT PROCESSES

As shown in Table 3, conventional domestic wastewater treatment processes may not be totally effective in inactivating and/or destroying parasites.

TABLE 3. EFFECTS OF WASTEWATER TREATMENT PROCESSES ON PARASITE EGGS AND CYSTS¹

Unit Operation	Effectiveness	Sources
Removal Processes (No Parasite Destruction):		
Clarifiers (Primary and Secondary)	80% Removal of <u>Ascaris</u> 54% Removal of <u>Entamoeba</u> Removal depends on operating conditions	15 , 18
Flotation	>95% Removal but depends on egg state and operating conditions	60
Imhoff Tanks	97% Removal	61
Trickling Filtration	38% Removal Promotes egg development	62, 63
Filtration	Retained 99% of eggs	42, 62, 63
Stabilization Processes (Affecting the Eggs; State):		
Anaerobic Digesters	Retards egg development (Increases destruction with in- creased temperature)	60, 62, 63, 64
Activated Sludge	Promotes egg development	15, 18
Extended Aeration	Promotes egg development	15
Decontamination Processes (Possible Egg Destruction):		
Incineration	100% Destruction	
Vacuum Filtration	No Effect	
Centrifugation	No Effect	
Drying Beds	100% Kill at <5% moisture content	15
Composting	100% Effective if all matter reaches 60°C for at least 2 hours	65, 70
Routine Chlorination	No Effect	40, 42
Sonication	Possible but not effective on <u>Ascaris</u> at 800 kHz and 150 watts	41
Gamma Radiation	Not 100% Effective (depends on eggs' state)	47, 71

(continued)

TABLE 3. (continued)

Unit Operation	Effectiveness	Sources
Heat	100% Effective above 70°C for 30 minutes or in less time at higher temperatures. Effectiveness depends on temperature and exposure time but temperatures below 45°C appear to have no effect	42, 43, 70

¹ Primarily Ascaris eggs.

The conventional domestic wastewater treatment processes can be divided into three categories: 1) removal processes (remove contaminants from wastewater); 2) stabilization processes (decrease bulk organics, odor, and pathogen content of sludges); and 3) inactivation processes (make the handling and disposal of sludges safer and more economical). These three major categories also have been found to affect parasites in different ways. Because parasite eggs and cysts are relatively heavy as compared to water they are concentrated with solids. In general, these processes have been observed to be up to 90% effective in the removal of parasites with the one major exception being the trickling filter which actually does not filter the sludge. Parasite removal that does occur takes place in the clarifier following the trickling filter. In sludge stabilization processes, aerobic or anaerobic environments are produced which may or may not be heated to lethal temperatures. Because most parasite eggs and cysts require an oxygen level above that of the gut in the host for development, anaerobic digesters tend to retard their development while aerobic digesters tend to accelerate it. As expected these processes will kill the eggs if either the anaerobic or aerobic processes are carried out at temperatures which are lethal to parasites (>55°C). Some sludge dewatering and disinfection processes will destroy the eggs by increasing the temperature, as in incineration and composting, or by greatly reducing the moisture content, as in drying beds. In more exotic cases, eggs may be destroyed by disruption of their biological subunits with sonication, radiation, or microwaves. These three major treatment processes are examined in more detail in the following.

REMOVAL PROCESSES

The effectiveness of clarifiers for the separation of parasite eggs and cysts from aqueous supernatants depends upon their operation and design. If the clarifiers are either overloaded or have short circuiting, then parasite eggs can enter the effluent weirs along with solids. Under laboratory conditions sedimentation removed only 89% of Taenia saginata eggs present in sewage in a three hour period (63). In another study using a tank with a depth of 26 inches, it was found that 100% of the Ascaris lumbricoides eggs present in sewage settled to the bottom in 30 minutes, some hookworm eggs settled to less than 1/3 of the depth after 2 1/2 hours, and not all Entamoeba

histolytica cysts settled to the bottom in a three hour period (15). The liquid fraction removal efficiency of a chlorinated primary sewage treatment plant was reported to be 80% for Ascaris eggs and 54% for Entamoeba coli cysts (18).

Air flotation has also been shown to be another possible means of egg removal from the liquid fraction. Some workers have found the method to be 85% effective for the removal of Ascaris eggs (60), and other workers after studying the removal rate of Ascaris eggs have recommended that sewage intended for agricultural purposes should be treated with air flotation followed by sedimentation (72).

The use of sand filters has given varying results for the removal of parasite eggs and cysts from final sewage effluents. Newton et al. reported that a 12-inch column of sand removed over 99% of the Taenia saginata eggs that had been added to settled sewage (63). In the field studies of Silverman and Griffiths, however, it was found that only 50% of the Ascaris and T. saginata eggs present were removed, and it was stated that this low removal was perhaps due to filter particle size and the applied rate of flow (62). In another laboratory study, Lepak found that 84% of the Ascaris eggs and 35% of the Entamoeba coli cysts were removed by sand filtration (19).

Various biological processes have been studied to determine their effectiveness for inactivating parasites in domestic wastewaters. Trickling filter systems have indicated a poor capability for the removal of the cysts and eggs of parasites. Newton et al. reported that only 30 to 38% of Taenia saginata eggs were removed by a trickling filter in the laboratory (63). Cram showed in another trickling filter study that there was no correlation between the removal of BOD and the removal of Entamoeba histolytica cysts and four types of helminth eggs. This trickling filter was, however, more successful at removing cysts than eggs (15). The Imhoff process has been reported to retain 97% of the incoming parasite eggs due to clarification. Cram noted that amoebic cysts and helminth eggs could survive the activated sludge process irrespective of the mode of treatment (15). Field studies have shown that trickling filters, sand filters and the activated sludge processes promote embryonation of helminth eggs such as Ascaris, Necator and Ancylostoma (15,62,63).

SLUDGE STABILIZATION PROCESSES

Because the majority of eggs and cysts of parasites are generally incorporated into sludges, the effectiveness of commonly used stabilization processes such as aerobic or anaerobic digestion are of particular importance. Varied results have been reported as to the effectiveness of conventional anaerobic and aerobic sludge digestion on parasite inactivation.

Hays (22) reported that most workers have found a reduction in the density of parasite eggs and cysts during anaerobic digestion. Reyes et al. (43) added Ascaris suum eggs, taken from the uteri of worms, to human feces adjusted to 3% solids concentration with urine and then anaerobically digested batches of this mixture. After 45 days, 85, 89 and 92% of the eggs were inactivated in the tests conducted at 25°, 30°, and 38°C, respectively.

In most situations, aerobic digestion induces embryonation of parasite eggs (22,43,62). Reyes *et al.* found 30% and 1% of fresh parasite eggs developed to the embryonated stage in night soil within 20 days under aerobic conditions at 30°C and 40°C, respectively (43). At or above 45°C, inactivation and/or destruction of most parasite eggs occurred, and parasite eggs that were still intact were unable to develop. Over 98% of the parasite eggs were destroyed in 20 days at a digestion temperature of 45°C and within two hours at a temperature of 50°C.

In general, researchers have found a time-temperature correlation for parasite kill and biological digestion processes. At temperatures above 60°C, all parasite eggs and cysts will be inactivated within 30 minutes (22). A 100% inactivation of Ascaris lumbricoides eggs taken from feces has been observed in 60 minutes at 60°C and in 30 minutes at 65°C.

INACTIVATION PROCESSES

One very promising method of sludge disinfection is composting. Various studies have reported that composted sludges are free of viable parasites (65,67). In compost piles, temperatures as high as 87°C have been reported; however, temperatures in the center of compost piles are generally between 65°C to 71°C (22). In a study where the temperatures were maintained between 60°C to 70°C for three days, 100% destruction of Ascaris eggs was found (67). Other composting studies have found that much longer periods (2 to 5 weeks) are needed before complete destruction of parasite eggs is obtained even when temperatures in the compost piles' centers are greater than 60°C (68). Composting is capable of completely destroying eggs and cysts if temperatures of 55°C or above are maintained for at least three consecutive days, and provided that all of the compost pile including its toes is subjected to this temperature (70).

The resistance of parasite eggs and cysts to dessication is species dependent with Ascaris eggs being considered the most resistant. Until the moisture content of the sludge was below 98%, Cram (15) found hookworm larvae active and infective in sludge dried up to 62 days in drying beds. In a laboratory study, Cram (16) discovered viable Ascaris eggs in sludge which had been dried for 81 days to a moisture content of 5.8% in a greenhouse in which temperatures often reached 46°C. Under field conditions, E. histolytica cysts and Ascaris eggs on tomato surfaces survived three days and one month, respectively (16). Other investigations have indicated that Ascaris eggs die when the moisture content of the sludge is less than 5% (15).

Low temperatures seem to have little effect on the survival of some parasite eggs in soils. According to Rudolfs *et al.* (73), Owen noted that Toxocara canis eggs survived in the soil in Minnesota for a year even though the ground temperatures were as low as -14.9°C in the winter. In Siberia, Ascaris lumbricoides eggs survived for over seven years at soil depths of 10 to 20 cm

under severe winter conditions (74). Cram found that unembryonated eggs of A. lumbricoides survived for 40 days at -19 to -26°C but that fully embryonated eggs were killed by a 20-day exposure to -21 to -27°C (45).

Results of studies on the destruction of parasites in sludges by chemicals have been varied. Investigators report that chlorine and ozone dosages used for routine disinfection of sewage effluents and drinking waters have no adverse effect on parasite eggs or cysts (42). At free residual chlorine concentrations of 3.9 to 10.0 mg/l, Schistosoma japonicum eggs were destroyed (75). To affect a 100% destruction of S. mansoni eggs in a 30 minute contact period, dosages of 4.0 mg/l of chlorine, 22 mg/l of iodine, or 24 mg/l of bromine were required (25). As with other pathogens, there is an all-or-none effect of ozonation on S. mansoni eggs. For example, S. mansoni eggs in treated wastewaters were not affected at ozone dosages of 25 mg/l but all were destroyed at ozone dosages of 40 mg/l with a 5 minute residual ozone concentration of 1 mg/l (40). In dechlorinated tap water, an ozone dosage of 15 mg/l was ineffective against Schistosoma eggs even with a 5 minute residual concentration of 0.99 mg/l; yet, in another experiment in the same study this same dosage was completely effective in destroying Schistosoma eggs with a five minute residual concentration of 0.13 mg/l (40). The liming of sludge to pH 12, using 135 to 465 kg of lime as calcium oxide per cubic meter of sludge (depending on the quality and origin of the sludge) was ineffective in inactivating the eggs of Parascaris equorum (76). Russian workers have reported that ammonium hydroxide at a concentration of 5% of the sludge volume destroyed Ascaris lumbricoides eggs in the solid portion of sewage within 20 days at 18 to 22°C. At higher concentrations of ammonium hydroxide (8% to 12%), Ascaris lumbricoides eggs were also effectively inactivated in shorter contact periods; i.e., 3 to 5 days (77).

More exotic forms of disinfection have been investigated in recent years including the application of ultrasonics, irradiation (beta and gamma), microwave, and combinations of these methods with chemicals. The following results were obtained in a study on the inactivation of parasites by ozonation either alone or in combination with ultrasonication using a 150 watt output energizing an 800 kHz transducer (41):

- a) Nippostrongylus brasiliensis eggs were completely inactivated in phosphate buffered saline (PBS) after treatment with ozone for 1 minute (1.7mg/l residual ozone). Sonication completely inactivated the eggs in PBS after 15 seconds. N. brasiliensis eggs in secondary effluent required 6 minutes treatment with 0.5 mg/l residual ozone for complete inactivation. Sonication completely inactivated the eggs in 15 seconds.
- b) Treatment of Schistosoma mansoni eggs in PBS by ozonation resulted in complete inactivation after 8 minutes with more than 2.2 mg/l residual ozone. Ozonation and sonication completely inactivated the eggs in 3 minutes 15 seconds at 0.63mg/l ozone residual. Sonication and ozonation seemed to exhibit synergism, in that they both produced a kill separately, but were more effective when used in conjunction; S. mansoni eggs were completely inactivated in secondary

effluent by ozone in 2 minutes 45 seconds using 0.1mg/l of residual treatment. The more rapid inactivation was reported to be due to salts added to the secondary effluent which decreased the ozone bubble size

- c) Ascaris lumbricoides eggs taken directly from the uteri of worms, suspended in PBS (4.4mg/l ozone residual) or secondary effluent (1.1mg/l ozone residual) were resistant to treatment by ozonation and ozonation-sonication at 1.28mg/l residual ozone for 10 minutes. Also, little or no effect on Hymenolepis diminuta eggs was observed

Studies on the disinfection of an undefined sludge by a standard microwave oven showed that two minute exposure increased the temperature of the sludge to 70°C and resulted in 99% kill of Ascaris eggs (78). When gamma radiation dosages of 100 krad from a cobalt 60 source were applied to raw and digested sludge containing Ascaris eggs (85% were potentially infective) only 1.0 to 0.7% of the Ascaris eggs remained viable after exposure. Complete Ascaris inactivation did not occur even at 500 krad (71). Alexandre *et al.* reported that gamma radiation from a 12,000 Ci cobalt 60 source in dosages of 500, 800, 1200, and 1800 krad had no effect on Parascaris equorum (Ascaris megocephala) which still had their outer coat. However, all four dosages killed the P. equorum eggs without outer shells (47).

PROMISING APPROACHES FOR INACTIVATION OF PARASITES

Even though current sludge stabilization processes do not appear to be very effective in parasite inactivation, a thorough investigation of both aerobic and anaerobic digestion with respect to temperature and additional treatment processes should be investigated for the development of a rational approach for inactivating parasites in sludges. From ongoing research it would appear that parasite inactivation at ambient temperatures may be possible by the following techniques: 1) aerobic digestion at a proper food-to-microorganism ratio; 2) aerobic digestion with lime stabilization; 3) anaerobic digestion with lime stabilization and/or ammonia addition depending upon the free ammonia concentration; 4) lime stabilization; and 5) drying beds at varying moisture contents and temperatures.

Other approaches which may prove useful in inactivating parasites in domestic waste sludges are: 1) ultrasonics, 2) free ammonia, and 3) surface active agents. Potentially, the simplest and most economical technique may be ultrasonication. The power cost for the application of ultrasonics in the descaling of boilers and cooling towers has been found to be negligible. (Reimers, R.S., unpublished data, 1979). The literature (38, 39, 76, 78) reports ammonia to be effective in destroying Ascaris eggs, but this inactivation was only at very high ammonia concentrations (greater than 5%). In those studies, the acidic sewage sludges were neutralized either by anhydrous ammonia or ammonium hydroxide. A mixture of lime and the less hazardous ammonium sulfate should be less expensive and require lower ammonium levels for inactivation of parasites. Because sludges are usually utilized as a soil amender and not as often as a fertilizer due to its low nitrogen and phosphorous levels, the addition of ammonia for parasite inactivation will also increase nitrogen levels in

sludges. Since the addition of nitrogen to soils using sludge is required in many cases, the addition of ammonia at this point of sludge treatment would serve two purposes: 1) inactivation of parasites; and 2) addition of the needed nitrogen nutrient. A third approach for parasite inactivation is the application of surface active agents during aerobic digestion which allows the agent to serve a dual purpose: a) conditioning the waste sludge; and b) aiding in the destruction of helminth eggs.

SECTION 5
METHODOLOGY

FIELD STUDIES

The bases for selecting the wastewater treatment plants for parasite sampling were: 1) method of treatment; 2) size of the plant; 3) region served; and 4) geographical conditions. Table 4 shows the matrix for initial plant selection. The southern region of the United States was chosen due to the expectant high prevalence of parasites and variation in climate.

TABLE 4 CRITERIA FOR SELECTING WASTEWATER TREATMENT PLANTS

Plant Size		Plant Type	Climate	Sludge Stabilization
1-5	MGD	Activated Sludge or Trickling Filter	Humid/Arid	Aerobic or Anaerobic Digestion
10-20	MGD	Activated Sludge	Humid/Arid	Aerobic or Anaerobic Digestion
>20	MGD	Activated Sludge	Humid/Arid	Aerobic or Anaerobic Digestion

Initially information was obtained on municipal wastewater treatment plants in the five southern states of Texas, Louisiana, Alabama, Florida and Mississippi to aid in the selection of appropriate plants for analyses of sludges. Table 5 lists both wastewater treatment and sludge treatment processes examined for their effectiveness for inactivating parasites. Thirty-eight municipal treatment plants were chosen for the initial screening.

TABLE 5. WASTEWATER TREATMENT AND SLUDGE TREATMENT PROCESSES INVESTIGATED

Wastewater Treatment Processes	Sludge Treatment Processes
Primary Clarification	Aerobic Digestion (continued)

TABLE 5. (continued)

Wastewater Treatment Processes	Sludge Treatment Processes
Secondary Clarification	Anaerobic Digestion
Activated Sludge	Vacuum Filtration
Extended Aeration	Centrifugation
Trickling Filters	Lime Stabilization
Imhoff Tanks	Drying Beds

As a result of the initial screening, the number of plants to be examined was reduced from 38 to 27. The selection of the final 27 plants was based on the accessibility of the plant, the cooperation of the plant's staff and management, treatment processes (both wastewater and sludge), the balance desired to evaluate different systems, and the initial parasitological data obtained in this screening phase.

Once each season grab samples of undigested or raw sludge and samples of the oldest drying bed sludge were obtained and analyzed for parasites. If raw sludge samples were not possible to obtain, then sludge samples closest to a raw sludge sample (returned, extended, aerated sludge or five gallons of raw sewage) were sampled. Likewise, if a final drying bed sample could not be analyzed, then the final stabilized sludge (digested sludge, lagoon sludge, vacuum filtered sludge, or centrifuged sludge) was obtained for parasite analysis. During sludge sample collection, data were also obtained on the ambient temperatures, recent rainfall, age of stabilized sludge samples, and sources of raw wastewater entering the municipal system.

The collected samples were analyzed within one week for types of parasites present, densities (number/volume or weight), and parasite condition (viable or nonviable). These samples were further analyzed for total solids and percent moisture content so that the final concentration of parasite eggs could be converted to a dry weight basis for comparison purposes.

One treatment plant, containing an unusually high number of parasites, was examined for a mass parasite balance over the total wastewater system. The treatment processes in this plant consisted of a bar screen, grit chamber, contact stabilization basin, sludge stabilization tank, clarifiers, aerobic sludge digestion, and sand drying beds. This plant was sampled at peak and low flows for two consecutive days. The mass balance was carried out by sampling each process step at the end of its calculated hydraulic retention

time. These sampling sites were: raw sewage, mixed liquor at the beginning of contact stabilization, mixed liquor after contact stabilization, effluent from clarifiers, return sludge, and sludge after stabilization. All the sludge samples were analyzed for types, densities, and viability of parasites present, and suspended solids concentrations. The raw sewage influent samples were also analyzed for volatile suspended solids, total organic carbon, chemical oxygen demand, and Kjeldahl nitrogen according to Standard Methods (79).

Verification of Sampling Procedures

In order to ascertain the reliability of the method of obtaining grab samples from drying beds, extensive testing was run on a selected drying bed. Samples were taken at various locations in the drying bed, analyzed for various parasites, and the moisture content measured. The results obtained for Ascaris eggs are shown in Figure 1 and Table 6. Generally, the parasites in the drying bed were found to be distributed relatively randomly as long as the moisture content was similar ($\pm 10\%$). An important point was that a sample taken from any portion of the drying bed was representative of the parasite population in the entire drying bed. The only exception to this observation occurred when there was considerable difference in moisture content.

Parasitologic Technique for Field Studies

The approach to the development of a parasitologic technique for the examination for sewage sludges was as follows: 1) the literature was reviewed to determine the techniques used by other investigators for the recovery of parasites from feces, wastewater, soil, and sludge; 2) criteria were established for a satisfactory analytical technique; and 3) parasitologic techniques used for the examination of feces, wastewater, soil and sludge were systematically tested, modified and evaluated. Sludge samples from local New Orleans area wastewater treatment plants containing low to moderate numbers of parasites were used to test the various techniques.

The criteria established as guidelines for the development of an analytical method for parasitologic examination of sludges were: 1) examination of relatively large sludge samples should be possible so that the densities of parasites can be measured when present at low levels; 2) viability of parasites should not be affected by technique; 3) quantitation of parasites should be possible; 4) efficiency should be relatively high; 5) accurate identification and determination of viability of parasites recovered should be possible; and 6) technique should be simple and relatively inexpensive, using equipment and materials normally available in a wastewater laboratory.

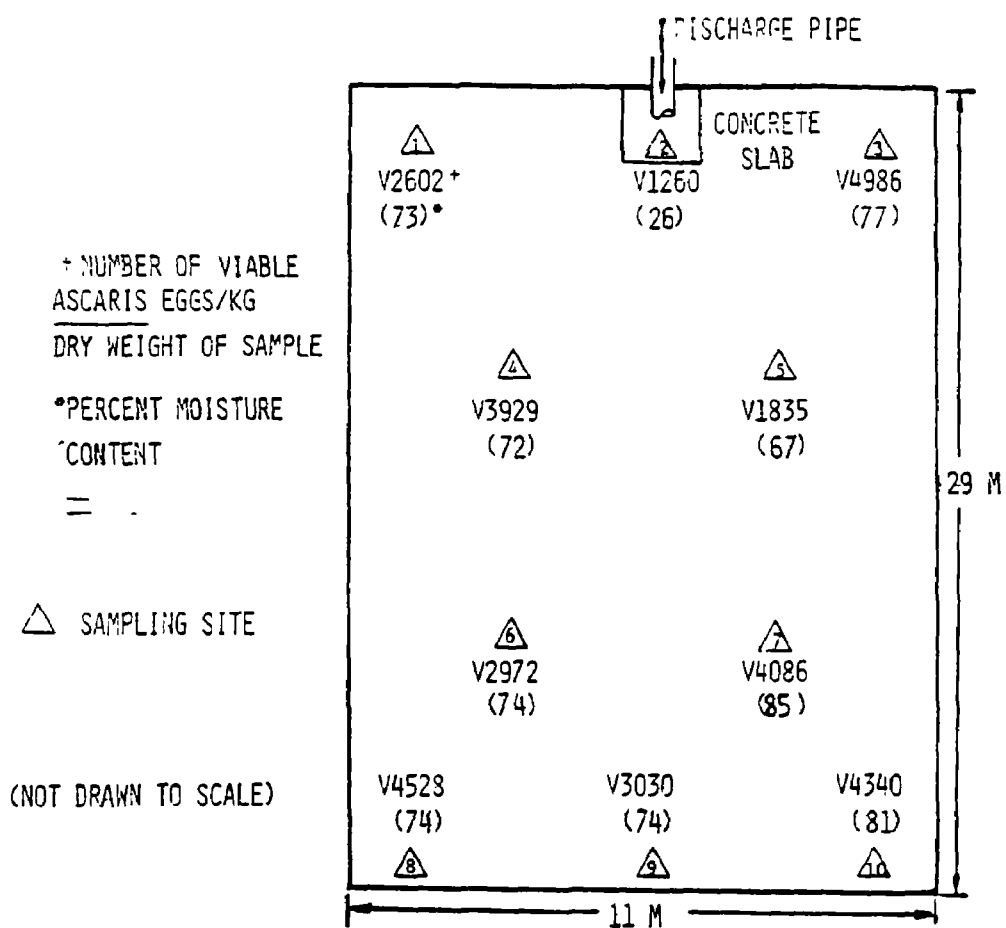


Figure 1. Statistical analysis of drying bed sample.

TABLE 6. STATISTICAL ANALYSIS OF DRYING BED SAMPLE

Parameter	Median	Average	Standard Deviation	Range
Moisture (%) Content	73.0	71.0	5.0	84.5-67.5
<u>Ascaris</u> eggs (No. of eggs/kg of sludge solids)				
Total	5170	4226	1572	6710-2950
Viable	3930	3590	1034	4986-1835
Non-viable	1080	946	417	1420-390
<u>T. trichiura</u> eggs (No. of eggs/kg of sludge solids)				
Total	630	638	258	990-260
Viable	480	483	242	860-130
Non-viable	130	154	111	310-0

Most concentration procedures that have been used for parasite recovery from feces, wastewater, soil, and sewage sludges use either sedimentation or flotation only (22, 80, 81). Others have used a sedimentation procedure in combination with a flotation procedure or other technique (18). One problem with a sedimentation technique (such as the one developed by Steer *et al.* (81)) is that a relatively small sample size of sludge (about 1 to 3 gms. wet weight) is used. Also, the sediment obtained contains relatively large amounts of material other than the parasites making it very difficult to find and identify the parasites in the sediment.

• Most investigators use flotation procedures for parasite analyses of sludges. The flotation procedures have the advantage of separating the parasites from the heavier particles in the sludges (sand, etc.) and consequently the final preparation is usually cleaner and easier to examine. In the flotation procedures, a solution is utilized with a specific gravity that is high enough to float the parasites from the sample but low enough to leave the heavier particles in the sediment. Solutions of sucrose, ZnSO_4 , NaCl , Na_2NO_3 , $\text{K}_2\text{Cr}_2\text{O}_7$ and other salts have been used. In the present study, an efficiency was evaluated for each of these different types of solutions. Based on the buoyancy of the different helminth eggs and protozoan cysts that might be present in sludge (82), it was concluded that the specific gravity of a solution used in the flotation procedure should be at least 1.20. In the present study, it was found that a solution of ZnSO_4 with a specific gravity of 1.20 was equal or superior to any of the other solutions tested. Dada and Lindquist (83) recently reported that a flotation solution of ZnSO_4 with a specific gravity of 1.20 was more efficient in recovering Toxocara eggs from soil than were solutions of $\text{Na}_2\text{Cr}_2\text{O}_7$ (sp. gr., 1.20), HgI_2 (sp. gr. 1.63) or ZnSO_4 with a lower specific gravity (1.18). Jackson *et al.* (24) used a ZnSO_4 solution with a specific gravity of 1.208 for the recovery of Ascaris eggs from sludges and vegetables.

Although the step involving the flotation and recovery of parasites from a sample is the main one in a flotation procedure, there are several other steps that affect the efficiency of the procedure. The initial step is usually one that involves the homogenization of the sample and the release of parasites adhering to other particles in the sludge. Steer *et al.* (81) found that by blending the sample in an anionic detergent both steps were accomplished. Meyer *et al.* (84) also found that the use of an anionic detergent improved the recovery of eggs after testing a variety of detergents (anionic, cationic, and nonionic).

A number of workers have used oxidants such as sodium hypochlorite to hydrolyze organic material and to release the parasites from other particles in the sludge (84). During this study sodium hypochlorite as well as other oxidants were tested, including perchloric acid and hydrogen peroxide, but each was found to have disadvantages. The problem encountered with sodium hypochlorite was that, if it is used in sufficient strength to be effective, it removes the outer layer of the shell of many of the helminth eggs. Since the structure and contours of the outer layer of the shell of certain eggs, especially those of *Ascaris* and *Toxocara*, are important in the identification of the eggs, the use of sodium hypochlorite can significantly affect the appearance of eggs and consequently the ability of the worker to identify them.

A step that can be used to remove extremely small particles (a few micrometers or less in size) from the sample is washing by gravity sedimentation. In this procedure, the sample is diluted in water or in anionic detergent and then allowed to settle. After settling for about one hour the supernatant which contains the fine particles in suspension is decanted.

Sieving is a procedure that can be effectively used to remove larger particles from the sample without removing parasites. After a series of tests, it was decided to use a step which involves passing the homogenized and washed sample through a series of graded sieves (10, 20, 50, 100 and 150 mesh). The openings on the final sieve (150 mesh) are about 106 μm which is large enough to allow eggs and cysts to pass through but small enough to retain many larger particles.

Several different procedures have been used for the recovery of eggs and cysts from the surface of the flotation solution. The use of a wire loop to remove the material floating on the surface is a commonly used technique (85). Meyer *et al.* (84) passed the flotation-solution supernatant through a membrane filter and then recovered the parasites from the surface of the filter. The procedure that was found to be very effective during this study was to decant the flotation-solution supernatant, add water to it until the specific gravity of the diluted solution is below that of the parasites, and then centrifuge this fluid to recover, in the form of a sediment, the parasites and other particles that had floated in the original flotation solution.

Fox used a different type of flotation procedure for detecting parasites in sludges, i.e., a continuous sucrose gradient (J.C. Fox, Personal Communication, 1977). The disadvantages of this technique are that only a relatively

small sample size can be processed in each tube and the apparatus used to form the continuous gradient is expensive and would not normally be available in a wastewater laboratory.

In the processing of some sludge samples, it was found that the amount of material recovered by the flotation procedure was too great to be easily examined microscopically. The particles of debris were so abundant that they obscured the parasites and made detection difficult. Also, the large amount of sediment recovered made the time required to microscopically examine the sample excessive. This problem led to attempts to find a method that could be used to further concentrate the parasites and eliminate most of the other particles. A procedure that is a modification of the acid-ether centrifugal sedimentation method (86) was developed. In this method, 0.27% H_2SO_4 in 35% ethanol (v/v) is used as the acid solution instead of the 5% acetic acid solution that is employed in the DeRivas method. With this technique it is possible to obtain a very clean preparation with a minimal amount of extraneous material present.

In developing a technique for the parasite analysis of sludges, it was found that the method used for the microscopical examination of the final preparation is extremely important. In order to detect small helminth eggs and the cysts of protozoa, the preparation requires examination under at least 100x magnification with a good compound microscope. In addition, to accurately identify some of the eggs and cysts and to determine viability it is necessary to examine them under even higher magnification, 400x or more. From our experience it is not possible to adequately examine preparations with a dissecting microscope.

The procedure that was finally adopted to use in the parasitologic examination of sludges from the municipal plants selected for study in this project is given in Appendix A. The efficiency of this technique was measured in several ways. Initially, it was estimated by examining sludges which had known numbers of Ascaris eggs added to them prior to incubation in anaerobic environments for a period of three weeks. The technique recovered 46 to 72% of the Ascaris eggs expected to be present in the samples (See Table A-1). However, the Ascaris eggs added to these test samples were eggs that had been obtained from the uteri of worms (Ascaris suum) recovered from the intestines of naturally infected pigs. Eggs obtained from this source tend to clump together and stick to glassware (24). One way to avoid this problem is to treat them with dilute sodium hypochlorite or sodium hydroxide which removes the outer layer of the shell. However, eggs that have had their outer coat removed are very much different from the Ascaris eggs that occur naturally in municipal sludges so this method was not employed. Consequently, many of the eggs added to test batches of the anaerobic sludge in the initial tests may have adhered to the walls of the glassware used in the experiment. For this reason it was concluded that the recovery of 50% or greater in most samples was quite satisfactory. An additional series of experiments were later run to test the efficiency of the technique on aerobically digested sludge and anaerobically digested sludge. In these tests, the Ascaris eggs added to the digestion flasks were eggs removed from the uteri of live worms and kept in the refrigerator for about 3 months prior to use. In Tables A-2 and A-3

it can be seen that the recovery of the added eggs from samples taken 25 days later was very low; 12% and 13% for anaerobic and aerobic samples, respectively. Undoubtedly, changes in the physical-chemical nature of the outer shell of the eggs during storage affected their behavior in the concentration procedure. However, it should be noted that in all three experiments the recovery of the eggs that occurred naturally in the sludge samples was consistent from one sample to another and did not decrease over the duration of the experiment. Also, it is indicated that the size of the sample examined and the number of eggs in the sample did not significantly affect the percent recovered.

The problems encountered in the use of Ascaris eggs from the uteri of worms for decontamination studies is discussed later in the Laboratory Methodology section. However, these problems dictated the use of Ascaris eggs obtained from the intestinal contents of naturally infected pigs for use in subsequent decontamination experiments. When eggs from this source were used, the concentration technique recovered a very high percentage of the eggs present in control samples. Over 90% of the theoretical number of eggs present in many of the aerobically digested control samples were recovered by the parasite analysis technique (Table C-2). Although the results obtained in analyzing "spiked" samples indicated that the technique employed was relatively efficient, it was not known whether the same efficiency could be obtained in recovering eggs that occurred naturally in sludge samples. Due to an extraordinary number of Ascaris eggs found in the drying bed sample from one plant (No. 7) it was possible to estimate the number of eggs present in the sample by another method, the Stoll dilution-egg-count-technique. In this technique, the sample is diluted in N/10 NaOH and the eggs present are counted directly in measured aliquot samples. By comparing the results from the Stoll Method with the results from our concentration technique it was estimated that our technique recovered about 83% of the eggs present in the sample tested.

Parasite Viability

The word viability is used in this study to indicate the state of being alive, i.e., a viable parasite is a parasite that is alive. Non-viable is used to indicate that a parasite is dead.

The determination of whether parasites recovered from municipal sludges and, in most cases, those recovered from experimental digesters were viable or non-viable was made on the basis of morphologic appearance. The morphologic criteria used to determine whether helminth eggs were viable or non-viable were similar to those used by several Japanese workers as reported by Morishita (26).

The morphologic changes appearing in eggs that were used as criteria of dead eggs included: 1) cytolysis of egg cell (ovum) or cells (after cleavage had started); 2) vacuolation of cytoplasm of ovum; 3) formation of large refractile granules within cell; 4) cytoplasmic hyalinization; 5) shrinkage of ovum; 6) disintegration of membrane surrounding ovum; 7) caving in of egg shell. In the case of eggs containing developing larvae viability or non-

viability was determined on the basis of appearance and whether the larvae showed movement after being stimulated by intense light from the microscope lamp.

The criteria used to indicate non-viability in protozoan cysts included: 1) shrinkage of cytoplasm from the cyst wall; 2) increased granulation of cytoplasm; 3) vacuolation in cytoplasm; 4) cytolysis; 5) karyorrhexis (fragmentation of nucleus); and 6) hyalinization of cytoplasm.

The reliability of the use of morphologic criteria to determine viability was evaluated in one series of experiments by culturing eggs after they had been examined microscopically. In experiments on anaerobic digestion and subsequent addition of ammonia (Tables E-8 and E-9), the eggs recovered from each sample were first examined microscopically and their viability determined on the basis of morphologic criteria. Then the eggs were washed from the microscope slide with distilled water into small petri dishes. They were covered with a shallow layer of distilled water (3-4 mm), stored at room temperature and agitated daily by rotating the dishes. After 2 to 3 weeks the material from each dish was removed and examined microscopically. Viability of cultured eggs was determined on the basis of whether or not larvae had developed within the eggs.

The results indicate that in most samples the estimation of viability of Ascaris eggs by morphologic criteria was fairly accurate (Tables E-8 and E-9). In the ammonia experiments, the method using morphologic criteria overestimated the number of viable eggs (as compared to the culture method) by only about 4% on the average. In the anaerobic digestion experiments run at 35°C, this method overestimated the viable eggs by less than 9% on the average.

In the anaerobic sludge study run at 35°C, the viability of Toxocara eggs as estimated by morphology was 9% higher on the average than the viability as determined by culture. However, in the ammonia experiments, the estimation of viability of Toxocara eggs on the basis of morphology was about 25% higher (average) than what was found by culture. This indicates that it is somewhat more difficult to differentiate between viable and non-viable Toxocara eggs on the basis of morphologic criteria than it is in the case of Ascaris eggs.

From test results obtained on sludge samples taken from the 45°C and 55°C anaerobic digesters it was observed that morphologic appearance alone was not reliable for judging the viability of parasite eggs (under lethal conditions). This is to be expected since the changes in morphologic appearance upon which non-viability is judged undoubtedly do not occur immediately. In the sample taken at 2 days from the 45°C digester none of the Ascaris or Toxocara eggs developed in culture, but 73% and 95% respectively, appeared to be alive on the basis of their morphology. In samples taken after 25 minutes from the 55 degree centigrade digester, the same error was observed, i.e., on the basis of morphology 9% of the Ascaris eggs and 53% of the Toxocara eggs were judged to be viable but by culture 0% and 1%, respectively, were viable.

LABORATORY PARASITE-INACTIVATION STUDIES

The laboratory studies on inactivation of parasites in municipal wastewater sludges were conducted in four phases. The effectiveness of aerobic and anaerobic digestion, lime stabilization, and ammonification on the inactivation of parasite eggs was investigated using sludges spiked with Ascaris suum and Toxocara canis eggs

Phase I - Aerobic Sludge Digestion

Sludge digesters were designed so they could be used for both aerobic and anaerobic digestion with only minor adjustment. Using a 45.7 cm length of 9.5 mm plexiglass tubing with an inner diameter of 25.4 cm, a digester of fifteen liter capacity was constructed. By using lids and bottoms of 9.5 mm thick plexiglass, the units were totally enclosed to retain parasite eggs for the safety of laboratory workers and for environmental control of the digester. Complete mixing was insured by using fan-cooled, heavy duty stirrers with two propellers on each shaft. Temperature was controlled with six feet of 25.4 mm wide heating tape connected in series to a rheostat and temperature controller which was connected to a temperature probe in the digester. Aerobic conditions were maintained by sparging air from the laboratory compressed air system through 15.2 cm airstones in the digesters. A control and a test reactor were run at each of the temperature conditions, i.e., ambient, 35°C, 45°C and 55°C. Seventy-five milliliter Kjeldahl bulbs were used to condense the water vapor in order to reduce as much as possible the evaporation loss due to aeration through the digester. The digesters were started with aerobic digested sludge from the Michoud Sewage Treatment Plant, New Orleans, Louisiana. Daily, a sample of one liter of digested sludge was removed from each digester, and one liter of a raw (return activated-primary) sludge mix (50/50 by volume) from the Helois Sewage Treatment Plant, Metairie, Louisiana, was added. Since a ten-liter working volume was utilized, a ten-day hydraulic retention time was assumed for aerobic digestion. Sludge parameters monitored included suspended solids, volatile suspended solids, pH, dissolved oxygen, and oxygen uptake rate. After the digesters obtained equilibrium, the reactors were spiked at time zero with Ascaris suum and Toxocara canis eggs. Samples were then collected every two days over a ten day period, and analyzed for types, concentrations, and conditions of parasite eggs. The efficiency of the digesters for parasite egg destruction was evaluated on the basis of the number of eggs recovered from the sample and the percentage of these that were viable.

Phase II - Lime Stabilization and Ammonification of Aerobic Digested Sludge

The effects of lime-stabilized aerobic-digested sludge on parasite eggs were examined using the aerobic-digested sludge and the surviving parasite eggs from the previous reactor experiment. Lime concentration of 0, 100, 1000 and 3000 milligrams of calcium hydroxide per gram of suspended solids were utilized depending on the lime's ability to maintain a pH greater than ten for fluctuating periods of time. The lime experiments were conducted over 20 days under both aerobic and anaerobic conditions.

The limed-aerobic-sludge experiment was effected by aerating 1700 ml of

digested sludge in plexiglass cylinders which were 45.7 cm in height by 15.2 cm I.D. with 12.7 mm thick walls. Air was supplied to the sludge by sparging compressed air through 25 4 mm long aquarium air stones. Complete mixing of the sludge was accomplished by using fan-cooled stirrers with two propellers on each shaft. The units were covered to reduce the release of aerosols that could contain parasite eggs.

The effect of lime stabilization under anaerobic conditions on Ascaris eggs were studied by adding various lime dosages to 800 ml of the aerobically digested sludge from the previous experiment. These limed sludges were placed in one liter screw-top plastic bottles and the bottles were shaken two to four times daily to mix the contents.

Samples were taken every five days and analyzed for parasite egg concentration, parasite viability, pH, suspended solids, and volatile suspended solids. Evaluation for the effectiveness of lime stabilization on parasite egg destruction was determined on the basis of percent viable eggs per sample and percent reduction in parasite eggs.

The influence of free ammonia on parasite egg destruction was also evaluated. Aerobically-digested sludge spiked with Ascaris suum and Toxocara canis eggs before digesting were employed. Varying concentrations of ammonium sulfate were added to 800 ml of sludge in air-tight, one liter, screw-cap plastic bottles in order to keep the generated NH_3 in contact with the sludge instead of allowing volatilization. At a lime dose of 3000 mg/l, concentrations of ammonium sulfate applied to these sludges were 0, 600 and 5000 mg per gram of suspended solids. The lime was employed to keep the pH greater than 10.4, which is above the pK_a (approximately 9.4) of NH_4^+ and NH_3 . By raising the pH to greater than 9.4, the predominant form of ammonia is the more toxic uncharged ammonia. The reactors were sampled every five days for 20 days and analyzed for NH_3 , suspended solids, volatile suspended solids, parasite egg concentration and viability.

Phase III - Anaerobic Sludge Digestion

The effect of continuous anaerobic sludge digestion on parasite eggs was investigated at various temperatures. The anaerobic digester design was similar to that for aerobic sludge digestion, except for the exclusion of air. A lead acetate bubbler was used following the Kjeldahl condenser to trap H_2S in the off gasses. The digesters were started up with anaerobic sludge from an operating anaerobic digester at the Camp Plaquemine Sewage Treatment Plant in New Orleans, Louisiana, and fed with primary and return activated sludge mix (50/50 by volume) from the Helois Sewage Treatment Plant. The digesters were fed one-and-a-half liters of feed sludge every two days. A 15-day hydraulic retention time was therefore obtained. Temperatures of ambient, 35°C, 45°C, and 55°C were maintained throughout the study period. The sludges were monitored for temperature, gas production, suspended solids, volatile suspended solids, pH, and total organic acids (volatile acids). After the digesters had reached equilibrium, Ascaris suum and Toxocara canis eggs were added. Samples were taken every second day for fifteen days (day 1, 3, 5, 7, etc.). The samples were analyzed for concentration and viability of parasite eggs, as

well as the previously mentioned physical and chemical parameters. Evaluation of the inactivation of parasite eggs was made on the same basis as that for the aerobic digestion experiments.

Phase IV - Ammonification of Anaerobic Digested Sludge

The influence of high free ammonia concentrations in anaerobically digested sludges on parasite eggs was examined. This experiment was carried out using the same methodology as elucidated in Phase II for the ammonification of aerobically-digested sludge; the anaerobic-digested sludge from the previous experiment was utilized. Sodium hydroxide was used instead of lime to raise the pH of the sludge above 10.4.

Phase V - Sonication of Parasite Eggs

This phase was concerned with the effects of contact time, sound wave frequency and intensity on the inactivity of Ascaris suum and Toxocara eggs. Sonic frequencies were controlled with 20, 50, and 80 kHz-sonic transducers, each with a wattage range of 0 to 100 watts.

Initially, the optimum wave frequencies and intensities for the destruction of Toxocara eggs in distilled water was ascertained. This study was carried out by exposing, in 200 ml beakers, Toxocara eggs in 100 ml of distilled water to 20 kHz and 10 watts for contact times from 30 seconds to 5 minutes. The samples were directly examined and evidence of parasite egg destruction noted. Subsequent runs were made in the same manner, with the exception that the wattage was increased 20 watts each time until a limit of 100 watts was attained.

The effects of sonication on Ascaris eggs was determined by the same procedure. The optimum wave frequencies and intensities found to destroy Toxocara eggs were employed to observe effects on Ascaris eggs. -----

Some of the samples in which there was no apparent egg destruction, yet which were exposed to wave frequencies and intensities near the optimum, were incubated for a one to two week period, then examined for possible embryo development.

PARASITOLOGIC TECHNIQUES

Test Organisms

In experimental studies on the effects of different sludge treatment procedures on parasites, two different test parasites were used, eggs of Ascaris suum and eggs of Toxocara canis. In the initial experiments, the A. suum eggs were obtained from the uteri of live worms that were recovered from the intestines of naturally infected pigs. The live worms were obtained from the Bryan Packing Co., West Point, Mississippi, and kept in a refrigerator until used. Usually within one or two days after they were received, the female

worms were opened and the anterior one-third of each uterus was removed. The collected pieces of uteri were placed in distilled water in a Waring blender and homogenized for about 30 seconds. The homogenate was then passed through several layers of cotton gauze to remove the larger particles. The resultant suspension of eggs was used immediately or was stored in the refrigerator until needed. The eggs that were added to test digesters were quantitated by the dilution-egg-count technique. A suspension of eggs was added to a Stoll-dilution-egg-count flask and measured aliquots of the thoroughly mixed suspension were transferred to microscope slides. The eggs in the aliquot sample were counted and the number of eggs per milliliter of suspension was then calculated.

Several problems were encountered when Ascaris eggs were obtained from the uteri of live worms for use in the initial efficiency testing of the parasite analysis technique. These eggs were very sticky and tended to clump together and to adhere to glassware. The clumping of the eggs made quantitation difficult since it was not possible to completely break up the clumps and obtain a homogenous suspension. This problem was also described recently by Jackson *et al.* (24). One method of preventing uterus-derived eggs from sticking together is to remove the outer layer of the shell. This can be accomplished by treating them with an aqueous solution of sodium hypochlorite (bleach) or sodium hydroxide. However, eggs which have been decoated in this manner have different shell characteristics from those that occur naturally in sewage sludges, and consequently, results obtained by using decoated eggs may be misleading.

The sticky nature of uterus-derived Ascaris egg is apparently due to the characteristic nature of the outer layer of the shell. According to Monné (87) the outer layer of the Ascaris egg within the uterus of a worm is a jelly-like coat that is composed of protein, mucopolysaccharides, and polyphenols. During or after the deposition of the eggs by the worm, this outer layer is hardened and tanned by the oxidation of the polyphenols to quinones, probably through an enzymatic reaction. An attempt was made to artificially harden and tan the outer shell of uterus-derived eggs but results were not successful. Eggs were exposed to bile salts, alkaline solutions, and alkaline solutions containing an oxidant and some success was achieved in hardening the outer shell but this only caused the eggs to clump together more firmly.

After it was determined that Ascaris eggs taken directly from the uteri of worms were not suitable for use as test organisms in the experimental studies (see discussion of laboratory studies on aerobic digestion), eggs were then obtained from the intestinal contents of naturally infected pigs. The contents of the intestines of infected pigs being processed at a slaughter house in Alabama were collected in large containers and transported to our laboratory in New Orleans. The intestinal contents were initially washed in tap water by repeated sedimentation and resuspension in water. After the supernatant was clear the sediment was passed through a series of graded sieves (10, 20, 30, 100 and 150 mesh) to remove the larger particles. The sieved material was stored in water in the refrigerator until used. The eggs in this suspension were quantitated by the procedure described above.

Toxocara canis eggs were obtained from the feces of naturally infected puppies. Fecal samples containing T. canis eggs (provided by a local veterinarian) were homogenized in tap water with the aid of a stirrer. The resulting fecal suspension was processed by the same method that was used for the fecal contents of pigs as described above.

EXAMINATION TECHNIQUES

The techniques used for the parasitologic examination of sludge samples from the experimental studies of aerobic and anaerobic digestion, lime stabilization, and ammonification were the same as those used for examining sludge samples from the municipal treatment plants (Appendix C) with the exception that the lime-stabilized sludges were neutralized before concentrating the parasites. In the initial lime stabilization experiments (Table E-4) each sample from the digester was mixed with about 900 ml of 0.2 N (H_2SO_4) to neutralize the lime and stop any further action on the parasite eggs. In samples containing higher lime dosages, this neutralization caused formation of large amounts of precipitate ($CaSO_4$). This precipitate made it difficult to process the specimen by the parasitologic technique and may have adversely affected the recovery of eggs. In subsequent experiments (Tables E-4, E-5 and E-6) the samples were neutralized with 0.1N HNO_3 and the problem of precipitate formation was eliminated.

SECTION 6

RESULTS AND DISCUSSION

INTRODUCTION

This research included both field and laboratory studies. The field studies consisted of a year-long investigation of parasites in domestic waste sludges in the southern United States. This investigation has resulted in new information concerning: 1) the types and concentrations of resistant stages of parasites in southern domestic sludge; 2) the seasonal fluctuation of these parasites in sludge; 3) the influence of climate on the parasites; 4) the effect of abattoir wastes on parasite content; and 5) other factors affecting the prevalence and persistence of parasites in sludges. Laboratory investigations were conducted on the effect of select sludge treatment processes on the inactivation of parasite eggs and cysts found in sludges. The treatment processes studied were aerobic and anaerobic digestion, lime stabilization, ammonification, sonication, and various combinations of these processes.

FIELD STUDIES

Parasitologic Findings

The results of the parasitologic examination of sludge samples collected from the 27 municipal plants are presented in Tables 7 through 12. It will be noted that many of the eggs or cysts of parasites found in sludges were identified only to genus or type. This is because the resistant stages of closely related parasites are often so similar that it is not possible to tell them apart. For example, the eggs of Ascaris lumbricoides and A. suum are indistinguishable and, consequently, when Ascaris eggs are found it is not possible to determine to which of these two species they belong. The probable identity of each type of helminth egg and protozoan cyst found in sludges is shown in Table 7.

TABLE 7. PARASITES FOUND IN SLUDGE SAMPLES FROM
27 MUNICIPAL PLANTS IN SOUTHERN UNITED STATES

Parasite Found	Probable Identity	Definitive Host
<u>Ascaris</u> eggs	<u>Ascaris lumbricoides</u> ¹	Humans
	<u>Ascaris suum</u> ¹	Pigs
<u>Toxocara</u> eggs	<u>Toxocara canis</u> ²	Dogs
	<u>Toxocara cati</u> ²	Cats

(continued)

TABLE 7. (continued)

Parasite Found	Probable Identity	Definitive Host
<u>Trichuris trichiura</u> eggs	<u>Trichuris trichiura</u> <u>Trichuris suis</u> ³	Humans Pigs
<u>Trichuris vulpis</u> eggs	<u>Trichuris vulpis</u>	Dogs
<u>Toxascaris</u> -like eggs	<u>Toxascaris leonina</u>	Dogs and Cats
<u>Ascaridia</u> -like eggs	<u>Ascaridia galli</u> <u>Heterakis gallinae</u>	Domestic poultry Domestic poultry
<u>Trichosomoides</u> -like eggs	<u>Trichosomoides crassicauda</u> <u>Anatrchosoma buccalis</u>	Rats Opossums
<u>Cruzia</u> -like eggs	<u>Cruzia americana</u>	Opossums
<u>Capillaria</u> spp. eggs (3 or more types)	<u>Capillaria hepatica</u> <u>Capillaria gastrica</u> <u>Capillaria</u> spp. <u>Capillaria</u> spp. <u>Capillaria</u> spp.	Rats Rats Domestic poultry Wild birds Wild mammals (opossums, raccoons, etc.)
<u>Hymenolepis diminuta</u> eggs	<u>Hymenolepis diminuta</u>	Rats
<u>Hymenolepis nana</u> eggs	<u>Hymenolepis nana</u>	Humans and rodents
<u>Hymenolepis</u> sp. eggs	<u>Hymenolepis</u> spp. (poss. more than one species)	Domestic and/or wild birds
<u>Taenia</u> sp. eggs	<u>Taenia saginata</u> ⁴ <u>Taenia pisiformis</u> ⁴ <u>Hydatigera taeniaeformis</u> ⁴	Humans Cats Dogs
<u>Acanthocephalan</u> eggs	<u>Macracanthorhynchus</u> <u>hirudinaceus</u>	Pigs
<u>Entamoeba coli</u> -like cysts	<u>Entamoeba coli</u> ⁵ <u>Entamoeba</u> spp.	Humans Rodents, etc.
<u>Giardia</u> cysts	<u>Giardia lamblia</u> <u>Giardia</u> spp.	Humans Dogs, cats, mammals
<u>Coccidia</u> oocysts	<u>Isospora</u> spp. <u>Eimeria</u> spp.	Dogs, cats Domestic and wild birds, mammals

TABLE 7. (continued)

- 1 Eggs of A. lumbricoides and A. suum are indistinguishable.
- 2 Toxocara eggs were probably mostly T. canis.
3. T. suis eggs probably only rarely seen.
4. Eggs of these worms are indistinguishable.
5. An intestinal amoeba that is a commensal, not a parasite.

TABLE 8. NUMBER OF MUNICIPAL PLANTS IN WHICH EGGS OF ASCARIS, TOXOCARA, TRICHURIS TRICHIURA AND TRICHURIS VULPIS WERE FOUND (27 PLANTS STUDIED)

Parasite	Fall ¹	Winter	Spring	Summer	Entire Year
<u>Ascaris</u>	17 ² /25 ³ /26 ⁴	22/25/26	14/26/27	14/25/25	26/27/27
<u>Toxocara</u>	11/22/24	17/27/27	9/24/24	9/23/25	23/27/27
<u>Trichuris trichiura</u>	6/10/16	8/12/18	7/10/19	6/10/16	12/15/26
<u>Trichuris vulpis</u>	19/21/22	19/23/24	19/23/26	12/24/25	25/26/27

- 1 Samples from only 26 plants examined in fall.
- 2 Number of plants in which viable eggs were found in treated sludges.
- 3 Number of plants in which viable eggs were found in any sludge sample.
- 4 Number of plants in which viable or non-viable eggs were found in any sludge sample.

TABLE 9. MISCELLANEOUS PARASITES FOUND IN SLUDGES FROM 27 MUNICIPAL TREATMENT PLANTS SAMPLED

Parasite	No. Plants in which found
<u>Toxascaris leonina</u> eggs	2
<u>Ascaridia</u> -like eggs	7
<u>Trichosomoides</u> -like eggs	7
<u>Cruzia</u> -like eggs	1
<u>Capillaria</u> eggs (shells with pits)	7

(continued)

TABLE 9. (continued)

Parasite	No. Plants in which found
<u>Capillaria</u> eggs (shell with striations)	11
<u>Hymenolepis diminuta</u> eggs	23
<u>Hymenolepis nana</u> eggs	6
<u>Taenia</u> sp. eggs	1
Acanthocephalan eggs	1
<u>Entamoeba coli</u> -like cysts	23
<u>Giardia</u> cysts	9
Coccidia oocysts	6

TABLE 10. AVERAGE NUMBER OF ASCARIS, TOXOCARA, TRICHURIS TRICHIURA AND TRICHURIS VULPIS EGGS FOUND IN PLANTS IN EACH GEOGRAPHIC REGION IN EACH SEASON

Geographic Region ² (No. of Plants)	Season	Parasite Eggs							
		<u>Ascaris</u>		<u>Toxocara</u>		<u>Trichuris trichiura</u>		<u>Trichuris vulpis</u>	
		Average ³	Standard	Average	Standard	Average	Standard	Average	Standard
			Deviation		Deviation		Deviation		Deviation
I (8)	Fall	2,100	4,200	800	1,100	100	300	300	500
	Winter	1,600	3,100	600	600	100	200	100	200
	Spring	1,700	3,400	600	1,300	600	1,100	400	500
	Summer	2,700	8,100	500	1,000	300	400	200	500
II (5)	Fall	12,900	16,000	900	1,100	7,000	13,600	1,100	1,600
	Winter	12,200	14,000	700	800	4,200	10,400	1,000	1,100
	Spring	10,700	11,000	800	1,300	12,300	27,100	1,400	1,100
	Summer	7,000	7,000	800	1,700	3,200	4,300	800	1,200
III (5)	Fall	3,500	5,300	900	1,300	100	200	300	600
	Winter	2,900	4,100	1,300	1,000	100	200	700	600
	Spring	1,800	3,600	1,000	600	100	300	400	500
	Summer	1,600	1,900	900	1,500	100	100	700	800
IV (7)	Fall	3,300	3,700	900	1,300	100	500	200	300
	Winter	6,600	7,300	2,200	1,200	1,100	2,700	1,600	3,100
	Spring	5,500	8,200	2,000	1,900	2,800	7,700	1,300	1,600
	Summer	11,500	14,400	1,700	2,500	900	2,200	1,000	1,300

1 Plant number 7 excluded.

2 See Figure 2 for description of geographic regions.

3 Average no. eggs/kg dry weight of sludge. Numbers rounded off to nearest 100. Includes viable and non-viable eggs in primary, secondary or treated sludges.

TABLE 11. AVERAGE NUMBER OF VIABLE EGGS OF ASCARIS, TOXOCARA, TRICHURIS TRICHIURA AND TRICHURIS VULPIS FOUND IN RAW SLUDGES (PRIMARY AND SECONDARY) IN PLANTS IN EACH GEOGRAPHIC REGION IN EACH SEASON¹

Geographic Region ²	Season	Parasite							
		<u>Ascaris</u>		<u>Toxocara</u>		<u>Trichuris trichiura</u>		<u>Trichuris vulpis</u>	
		spp.	Standard	spp.	Standard	Average	Standard	Average	Standard
		Average ³	Deviation	Average	Deviation		Deviation		Deviation
I	Fall	3,600	5,700	1,600	1,000	100	400	400	600
	Winter	2,800	4,100	1,100	600	100	300	100	100
	Spring	1,800	2,900	1,100	1,800	700	1,100	600	700
	Summer	5,100	11,300	1,000	1,200	600	500	400	400
II	Fall	7,500	4,600	1,300	1,300	800	1,000	600	700
	Winter	12,800	8,000	1,100	1,000	500	500	1,600	1,500
	Spring	10,900	7,200	300	200	3,700	3,300	1,600	1,000
	Summer	9,200	5,500	100	100	2,200	4,000	700	600
III	Fall	3,100	5,100	900	1,200	0	0	100	100
	Winter	3,200	4,200	1,600	700	0	0	500	400
	Spring	3,100	5,100	800	500	0	0	500	500
	Summer	2,400	2,500	1,700	2,000	0	0	500	500
IV	Fall	4,400	4,500	600	900	0	0	100	200
	Winter	7,800	8,300	3,200	1,300	200	200	900	1,200
	Spring	9,400	11,100	2,800	900	5,800	11,300	1,800	2,200
	Summer	11,100	13,300	1,300	1,100	200	400	1,400	1,700

1 Plant No. 7 excluded.

2 See Figure 2 for description of geographic regions.

3 Average number of viable eggs/kg dry weight of sludge. Numbers rounded off to nearest 100.

TABLE 12. AVERAGE NUMBER OF VIABLE EGGS OF ASCARIS, TOXOCARA, TRICHURIS TRICHIURA, AND TRICHURIS VULPIS FOUND IN TREATED SLUDGES IN PLANTS IN EACH GEOGRAPHIC REGION IN EACH SEASON¹

Geographic Region ²	Season	Parasite							
		<u>Ascaris</u>		<u>Toxocara</u>		<u>Trichuris trichiura</u>		<u>Trichuris vulpis</u>	
		spp.		spp.					
		Average ³	Standard Deviation	Average	Standard Deviation	Standard Deviation		Average	Standard Deviation
I	Fall	600	600	100	100	100	100	200	200
	Winter	400	500	100	100	100	100	200	200
	Spring	1,700	4,000	100	300	500	1,200	200	200
	Summer	200	400	100	100	100	200	100	200
II	Fall	17,200	21,700	700	1,000	11,900	17,200	1,400	2,100
	Winter	11,700	18,400	400	500	7,100	13,900	1,000	500
	Spring	10,600	14,800	1,200	1,700	19,200	36,400	1,300	1,200
	Summer	5,300	8,400	1,300	2,300	4,100	4,800	1,000	1,600
III	Fall	3,800	5,900	1,000	1,600	200	300	600	700
	Winter	2,700	4,500	1,100	1,200	200	200	800	700
	Spring	600	1,400	200	400	200	400	200	500
	Summer	900	1,000	300	500	100	100	800	900
IV	Fall	2,500	3,000	1,200	1,600	300	600	300	300
	Winter	5,600	7,100	1,400	2,700	1,900	3,600	2,200	4,100
	Spring	2,200	2,600	1,300	2,300	300	400	900	800
	Summer	11,100	13,300	2,000	3,300	1,500	2,900	700	800

1 Plant No. 7 excluded.

2 See Figure 2 for description of geographic regions.

3 Average number of viable eggs/kg dry weight of sludge. Numbers rounded off to nearest 100.

As shown in Table 7, eggs identified as Toxocara were in most cases probably those of T. canis, the dog roundworm. The egg of T. cati, the cat roundworm, is very similar to that of T. canis but can be distinguished from the latter by the size of the pits in the surface layer of the shell when viewed under high magnification of the compound microscope. Because it would have been an extremely tedious and time consuming task to have made a specific identification of every Toxocara egg found, this was not attempted. However, the impression gained from examining these samples was that most of the Toxocara eggs found were those of T. canis. A similar situation exists in the case of the eggs of Trichuris trichiura, the human whipworm, and the eggs of T. suis, the swine whipworm, which are difficult to differentiate from each other. However, the impression was that only a small percentage of the T. trichiura-like eggs observed were those of T. suis.

Ascaris, Toxocara, Trichuris trichiura, and T. vulpis were the most commonly encountered parasites. The occurrence of these parasite eggs in sludges from the municipal plants sampled is summarized in Table 8. The eggs of Ascaris, Toxocara and Trichuris vulpis, either viable or non-viable, were recovered one or more times from each plant studied. Eggs of T. trichiura were found in all but one of the plants. Viable eggs of Ascaris and Toxocara were recovered at least once from every plant, and viable eggs of T. vulpis and T. trichiura were recovered at least once from 26 and 15 plants, respectively.

In Table 9, the other parasites, viable or non-viable, found in the sludges are shown. Of these, Hymenolepis diminuta was most frequently found; its eggs were found in 23 of the 27 plants studied. Viable eggs of H. diminuta were found in primary sludges in 15 plants and in treated sludges in 4 plants. H. diminuta is a tapeworm of rats, and its presence in sludge in 23 plants is an indication of the frequent occurrence of rats in or near sewerage systems and treatment plants. Other parasite eggs that are most likely to be from a rodent source include the Trichosomoides-like eggs and some of the Capillaria eggs. Hymenolepis nana eggs could have been from either humans or rodents.

Of the protozoan cysts found in sludge samples Entamoeba coli-like cysts were found most frequently. However, in only one sample, activated sludge from plant 22, did any of these cysts appear to be viable. Since the cysts of E. coli are more resistant to environmental conditions than are the cysts of Entamoeba histolytica, the pathogenic amoeba of humans, it is unlikely that any cysts of E. histolytica could, if present, pass through a sewage treatment plant or survive sludge treatment in a viable condition. E. coli-like cysts were found in samples from 5 plants in the fall, 18 plants in the winter, and 11 plants in each of the spring and summer collections. None of the Giardia cysts found in any sample appeared to be viable. Giardia cysts were found in samples from 2, 3, 4, and 5 of the 27 plants examined in the fall, winter, spring and summer, respectively.

None of the coccidian oocysts recovered appeared to be those of Toxoplasma gondii, the cause of toxoplasmosis in humans. However, the oocysts of this protozoan are only 10 x 12 micrometers in size so it is possible that

they were present in some samples but were overlooked in the examination.

Tables 10, 11, and 12 present data on the levels of eggs of Ascaris, Toxocara, Trichuris trichiura, and T. vulpis in plants within each geographic region as defined in Figure 2. Since the levels of Ascaris eggs in one plant (number 7) were so very high due to abattoir waste (as explained later), the data on this plant were not included. It can be seen that the human parasites Ascaris and T. trichiura, were found in higher numbers during all seasons in the sewage treatment plants sampled in Regions II and IV as compared to the plants sampled in Regions I and III. Regions II and IV include the coastal areas of the five states studied (See Figure 2). Due to the climate of this region (high humidity and temperature) the potential of human parasite survival is very possible. The levels of the eggs of Toxocara and T. vulpis, parasites of dogs (mostly); do not vary much either from one season to another or in the different regions.

The higher density levels of the Ascaris eggs as compared to other parasites are at least partly due to the higher fertility rate of this worm. The female of Ascaris may produce over 200,000 eggs each day (24,51), whereas the female of Trichuris or Toxocara produces only about 10,000 eggs per day or less.

Densities of Parasites in Sludges

The total number of parasite eggs recovered from sludge samples in all plants ranged from 0 to more than 230,000 eggs/kg dry weight of sludge, depending on the parasites involved, source of sludge and specific season. The average number of total parasite eggs present was found to be approximately 14,000/kg dry weight of sludge. The percentage of viable parasite eggs in each sample ranged from 0% to 100%, but was generally greater than 45%. Even though the concentration of total parasite eggs fluctuated over a wide range, observations can be made as to the number of specific parasites which can be expected for any given sludge as demonstrated in Table 13. Primary and secondary undigested sludge samples were found to contain, in order of decreasing average concentrations: 9,700 Ascaris spp. eggs, 1,200 Toxocara spp. eggs, 800 T. trichiura eggs, and 600 T. vulpis eggs/kg dry weight of sample. However, the average numbers of these parasites in stabilized sludge samples were found to be: 9,600 Ascaris spp. eggs, 2,600 T. trichiura eggs, 700 Toxocara spp. eggs, and 700 T. vulpis eggs/kg dry weight of sludge sample. Other parasite eggs and cysts were observed in the sludge samples, but in low concentration. Quantities of these four most prevalent parasites fluctuated greatly as illustrated by the fact that the standard deviations of their densities are greater than the observed averages and the ranges are from zero to 10 times the average.

It is difficult to compare the levels of helminth eggs found in domestic sewage sludges in the southern United States to those found in sludges in foreign countries due to the different procedures used in examining the sludges and the way the results are reported. In most cases, the reports from foreign countries, as well as most reported from the United States, give the number of eggs found per volume of liquid waste (liter, gallon, etc.) or

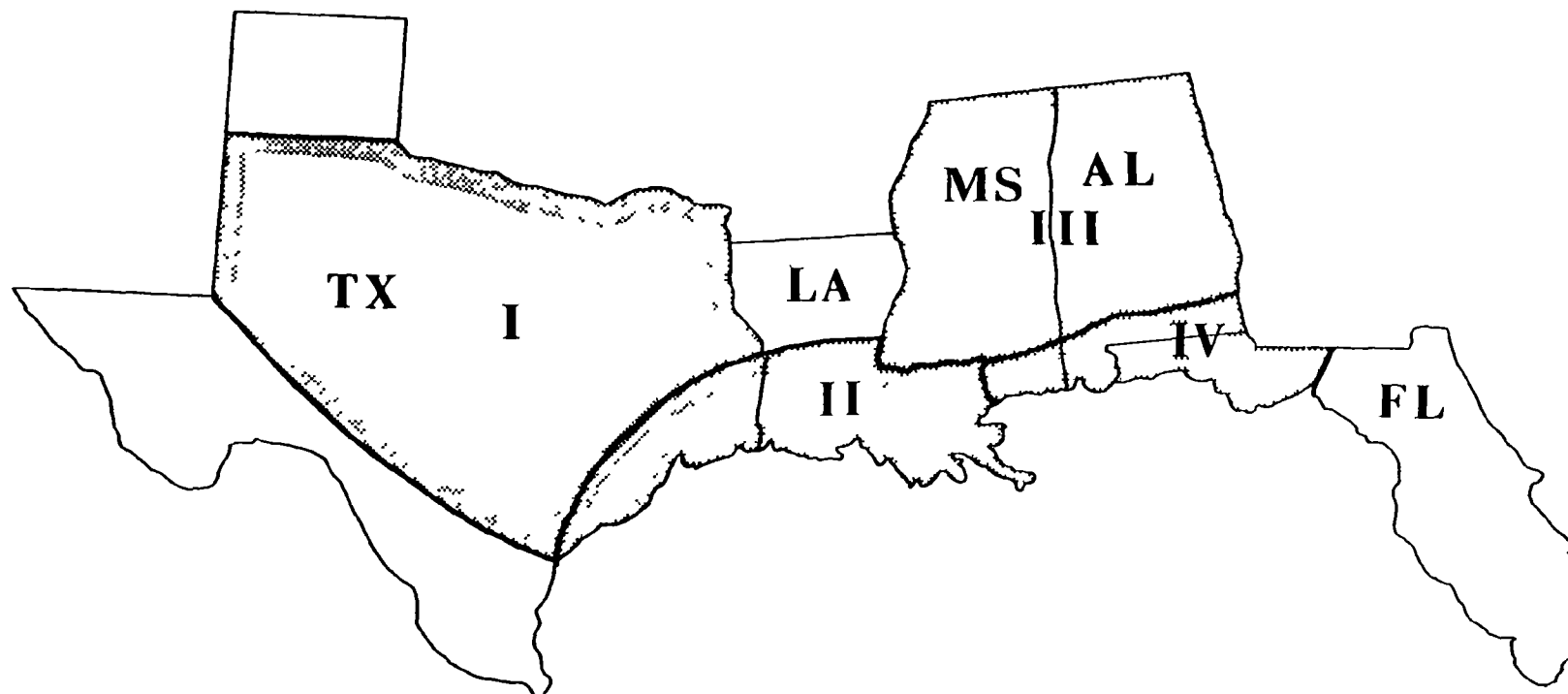


Figure 2. Geographic regions in which municipal waste treatment plants were studied.

TABLE 13. PARASITE CONCENTRATIONS IN PRIMARY AND SECONDARY SLUDGE AS
COMPARED TO TREATED SLUDGE

Parasite	Nature of Sludge ¹	Number of Viable and Non-viable Eggs/kd Dry Weight of Sample			Percent Viable Eggs
		Average ²	Standard Deviation	Range	
<u>Ascaris</u> spp. (human and pig roundworm)	Primary and Secondary	9,700	26,300	200,000 - 0	45
	Treated	9,600	27,400	230,000 - 0	69
<u>Trichuris</u> <u>trichiura</u> (human whipworm)	Primary and Secondary	800	2,900	26,000 - 0	50
	Treated	2,600	9,800	84,000 - 0	48
<u>Trichuris vulpis</u> (dog whipworm)	Primary and Secondary	600	1,000	5,700 - 0	90
	Treated	700	1,300	10,500 - 0	64
<u>Toxocara</u> spp. (dog and cat foundworm)	Primary and Secondary	1,200	2,300	5,400 - 0	88
	Treated	700	1,500	8,500 - 0	52

1 Primary and Secondary sludges include sludges from primary clarification, Imhoff digestion, activated sludge, contact stabilization, and extended aeration. Treated sludges include sludges from mesophilic aerobic and anaerobic digestion, vacuum filtration, centrifugation, lagoons and drying beds.

2 Numbers rounded off to nearest 100.

wet weight (g, kg, etc.) and do not give the dry weight or amount of solids in the samples. However, it appears that the levels of helminth eggs in southern United States sludges are, in general, lower than those that have recently been reported from certain foreign countries although the sludges from a few of the southern United States plants had comparable levels.

In 1976, Sadighian *et al.* (88) reported finding 1,000 to 13,000 Ascaris eggs per gram of raw sewage and 14,000 to 25,000 Ascaris eggs per gram of processed sludge in the sewage treatment facilities in Isfahan, Iran. They also found 500 to 1,500 Trichuris eggs per gram of raw sewage. This appears to be one of the highest levels of helminth eggs ever reported in sewage sludge.

In municipal wastes of Łódź, Poland, Gadomska *et al.* (89), in 1975, found 428 helminth eggs (Ascaris, Trichuris, Toxocara and Taenia) per kilogram of raw municipal sewage. In Smolensk, U.S.S.R., Asvin and Lagutina (90), in 1976, found an average of 5.7 eggs of Ascaris and Trichuris per liter of raw sewage and 60 eggs per kilogram of sewage sediment.

In Plant No. 7, which had the highest levels of Ascaris eggs of the southern United States plants studied in this project, an average of about 1,800 Ascaris eggs per liter was found in primary sludges and an average of about 45 Ascaris eggs per gram (wet weight) was found in drying bed sludges. In another southern plant (No. 18), as many as 1,200 Ascaris and 600 Trichuris trichiura eggs per liter of primary sludge and as many as 14 Ascaris and 10 T. trichiura eggs per gram (wet weight) of drying bed sludges were found. Most other United States plants were well below these levels.

The Influence of Abattoirs on Parasite Concentration

Of the plants included in this study only one, plant number 7, was found to have very high levels of Ascaris eggs in the sludges. Further study of this plant revealed that there was an abattoir in the community that processed large numbers of swine and that the wastes from the plant entered the municipal sewer system. In Table 14, the levels of parasite eggs in the sludges from this plant are compared with 6 plants of similar size from the same geographic area (Area IV). In the plant receiving abattoir wastes (plant number 7) an average of 81,800 Ascaris eggs/kg dry weight of sludge was recovered while in the 6 plants receiving little or no abattoir wastes an average of only 7,900 Ascaris eggs/kg dry weight sludge was recovered. The Ascaris eggs entering plant number 7 were undoubtedly mostly A. suum eggs that came from infected swine processed in the abattoir. It is interesting that the level of the T. trichiura-like eggs was nearly the same in each plant. This would indicate that few if any of these eggs were those of T. suis, the swine whipworm. Toxocara eggs densities were slightly lower in sludges from plant number 7 as compared to the other plants; but it is doubtful that this is significant.

Relationship of Treatment Plant Site to Parasite Concentrations

On the basis of the collected data, there was no correlation between egg

TABLE 14. INFLUENCE OF ABATTOIR WASTES ON PARASITE CONCENTRATIONS
IN PRIMARY AND SECONDARY SLUDGES

Parasite Eggs	Significant Source Contribution	Average No. of Viable and Non-Viable Eggs/kg Dry Weight of Sludge ¹	Number of Plants
<u>Ascaris</u> spp. (human and pig roundworms)	Domestic ²	7,900	6
	Abattoir ³	81,800	1
<u>Trichuris trichiura</u> or	Domestic	1,500	6
<u>Trichuris suis</u> (human or pig whipworms)	Abattoir	1,600	1
<u>Toxocara</u> spp. (dog and cat roundworms)	Domestic	1,800	6
	Abattoir	500	1

1 Numbers rounded off to nearest 100.

2 Domestic plants found in Geographic Area IV.

3 Treatment plant in Geographic Area IV receiving waste from large swine slaughter and packing houses.

densities and plant size. Even though the smaller plants serving more rural populations would be expected to have higher densities of parasites in the sludge, this phenomena was not observed.

Effects of Sludge Treatment Processes on Parasites

The results of this investigation on parasites in southern domestic sludges indicate that, in general, conventional sludge stabilization treatment processes (e.g., mesophilic anaerobic or aerobic digestion) are not completely effective in destroying parasite eggs (Table 15 and Figure 3). Drying beds, however, appear to be very effective in destroying parasites. The concentration processes of vacuum filtration and centrifugation appear to remove or destroy eggs but due to the low number of samples analyzed, this effect is somewhat questionable. Because these processes only remove water in a short processing period, there is little reason to expect them to significantly reduce the numbers of parasite eggs. In fact, the parasite eggs densities should increase during the dewatering process. One reason for lower concentrations may be due to the use of conditioners (inorganic electrolytes) that can bind eggs to sludge particles or destroy them indirectly by changes in pH, dissolved oxygen, oxidation-reduction potential, ammonium levels, etc.

In the field investigation, there was a great deal of data collected on influent raw sludges and drying bed treated sludges were the only stabilization of these sludges was either aerobic or anaerobic digestion under ambient or mesophilic temperatures. These results are shown in Table 16. During winter and fall, parasite inactivation tended to be most variant. In Figure 4, the reduction in numbers of the four predominant parasite eggs is plotted with respect to the drying bed process with no differentiation between aerobic and anaerobic stabilization. Except for Toxocara, the densities of all parasite eggs were reduced to a greater extent in the summer and the spring than they were in the fall and winter. Figures 5 and 6 indicate the influence of anaerobic and aerobic digestion on drying bed treatment for parasite eggs. A reduction in effectiveness during the winter and fall was noted with anaerobically digested sludges, yet with aerobically digested sludges, a seasonal influence was noted only with T. trichiura.

Although it would appear from these data that the eggs of Trichuris trichiura and T. vulpis are more resistant to sewage treatment processes than are the eggs of Ascaris and Toxocara, this may not be true. The viability of all eggs in this study was judged on the basis of morphologic criteria as described in an earlier section. It is possible that this method is less reliable for determining the state of viability of the eggs of Trichuris than for Ascaris or Toxocara. If this is true, then it could affect the results presented here. While good correlation was obtained between the estimation of the viability of Ascaris eggs by the method of using morphologic criteria and the method of culturing the eggs as reported elsewhere in this study, there was no opportunity to compare the two methods for estimating the viability of Trichuris eggs. Skrjabin

TABLE 15. AVERAGE PERCENT REDUCTION OF VIABLE PARASITE
EGGS BY UNIT PROCESSES¹

Process	<u>Ascaris</u>	<u>Toxocara</u>	<u>Trichuris</u> <u>trichiura</u>	<u>Trichuris</u> <u>vulpis</u>	
Aerobic Digestion	6 (121) 130	9 (167) 82	1 100 -	7 11 100	Number of Plants Reduction % Standard Deviation
Anaerobic Digestion	6 (118) 109	3 (36) 171	4 (97) 204	5 12 105	Number of Plants Reduction % Standard Deviation
Drying Bed (After Aerobic Digestion)	6 75 57	8 96 9	- - -	6 77 41	Number of Plants Reduction % Standard Deviation
Drying Bed (After Anaerobic Digestion)	5 97 3	4 97 6	1 100 -	5 93 9	Number of Plants Reduction % Standard Deviation
<u>Dewatering</u>					
Thickening	3 (110) 222	3 6 55	- - -	- - -	Number of Plants Reduction % Standard Deviation
Vacuum Filtration	3 61 27	3 (10) 109	1 100 -	3 44 79	Number of Plants Reduction % Standard Deviation
Centrifugation	2 37 71	3 53 67	1 100 -	2 62 54	Number of Plants Reduction % Standard Deviation

¹() = Negative reduction, i.e., percent increase

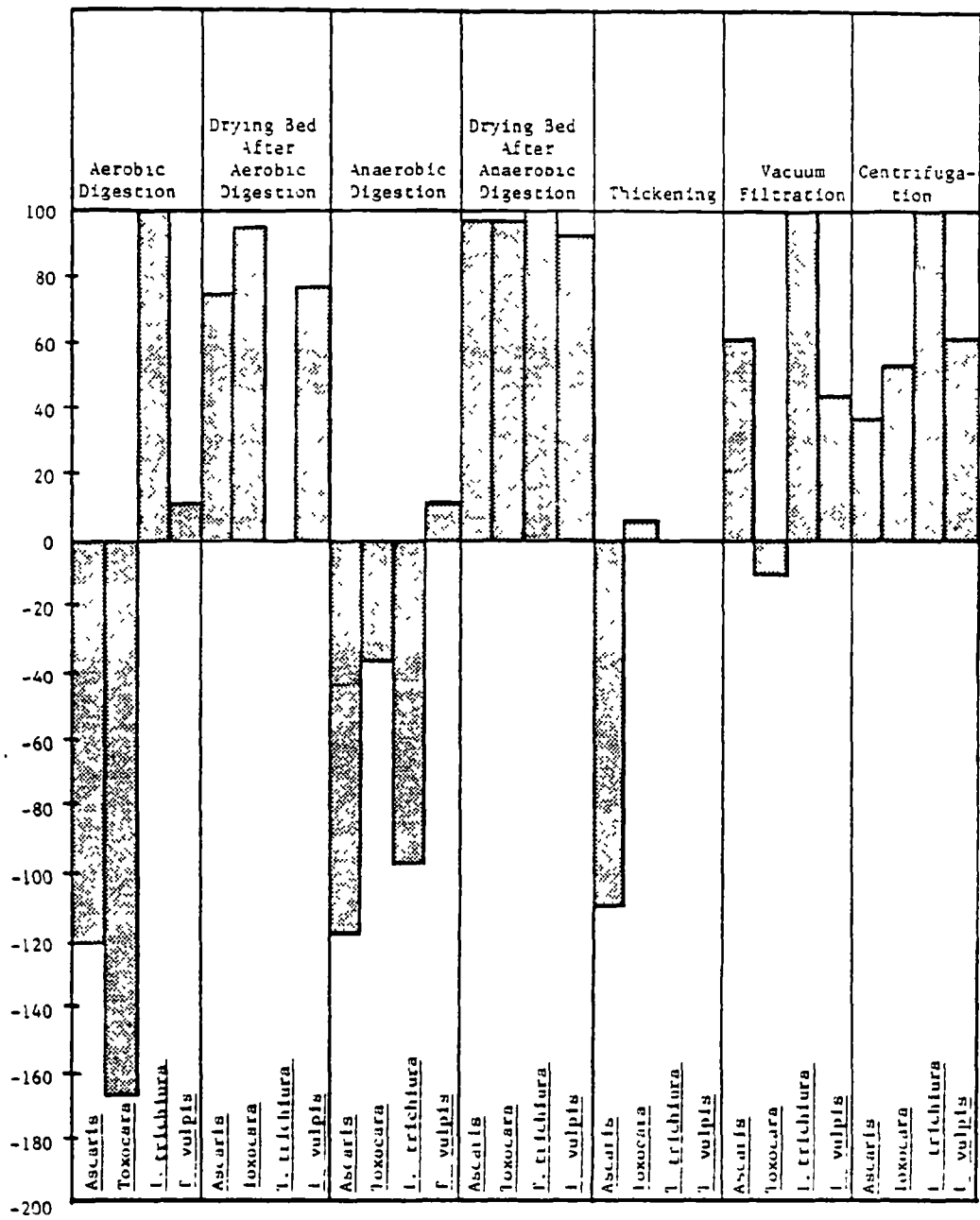


Figure 3. Average percent reduction of viable parasite eggs by unit process
(Negative values indicate that densities of parasites increased after the process.)

TABLE 16. PERCENT REDUCTION OF TABLE PARASITE EGGS BY
TOTAL SLUDGE TREATMENT PROCESSES¹

Process (Period)	<u>Ascaris</u>	<u>Toxocara</u>	<u>T. trichiura</u>	<u>T. vulpis</u>
Total	67/76/69 ²	91/28/74	39/118/26	44/87/58
Fall	53/101/16	82/39/16	31/87/7	(1) ³ /135/8
Winter	36/108/17	89/37/22	(26)/196/7	7/103/16
Spring	87/16/18	95/14/19	80/28/6	70/41/18
Summer	84/26/18	98/6/17	93/15/5	74/49/16
Aerobic	70/45/13	90/21/16	(25)/105/4	63/46/11
Fall	97/5/3	69/34/3	(66)/-/1	81/-/1
Winter	34/60/4	98/31/4	(155)/-/1	48/74/2
Spring	82/37/4	88/25/5	54/-/1	63/44/4
Summer	80/29/2	100/0/4	67/-/1	68/54/4
Anaerobic	66/83/55	91/31/55	52/119/21	34/96/43
Fall	49/111/13	83/42/12	48/82/6	(31)/145/6
Winter	37/121/13	86/42/17	(5)/206/6	(7)/113/13
Spring	89/24/14	98/6/14	85/27/5	71/42/13
Summer	84/28/15	97/7/12	100/0/4	74/51/11

1 Aerobic or anaerobic digestion followed by drying beds.

2 Average/standard deviation/number of samples.

3 Numbers in parentheses represent percent increase.

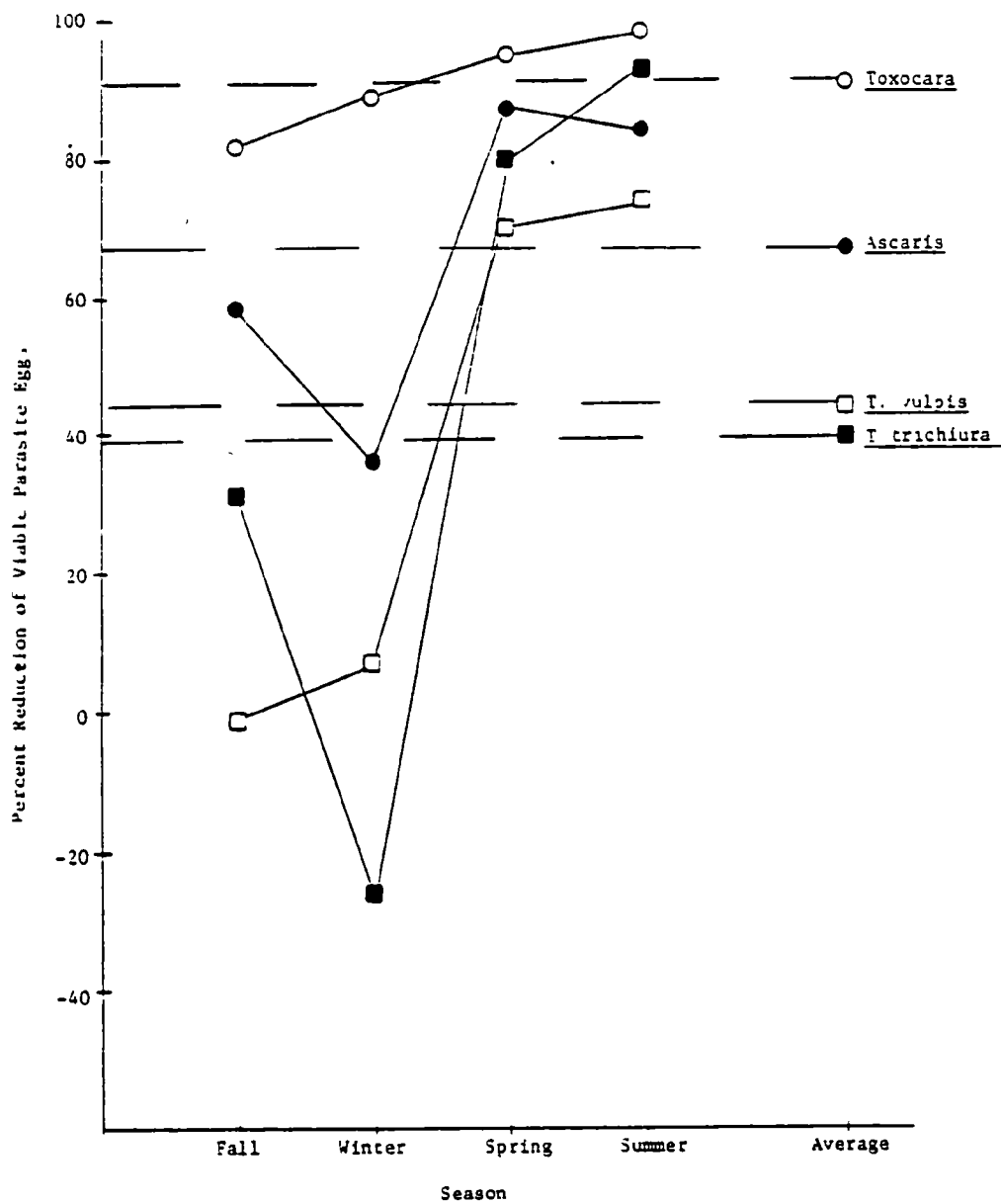


Figure 4. Percent reduction of viable parasite eggs in drying bed dewatered sludges following anaerobic or aerobic stabilization as compared to densities in raw sludge versus seasons and yearly averages.

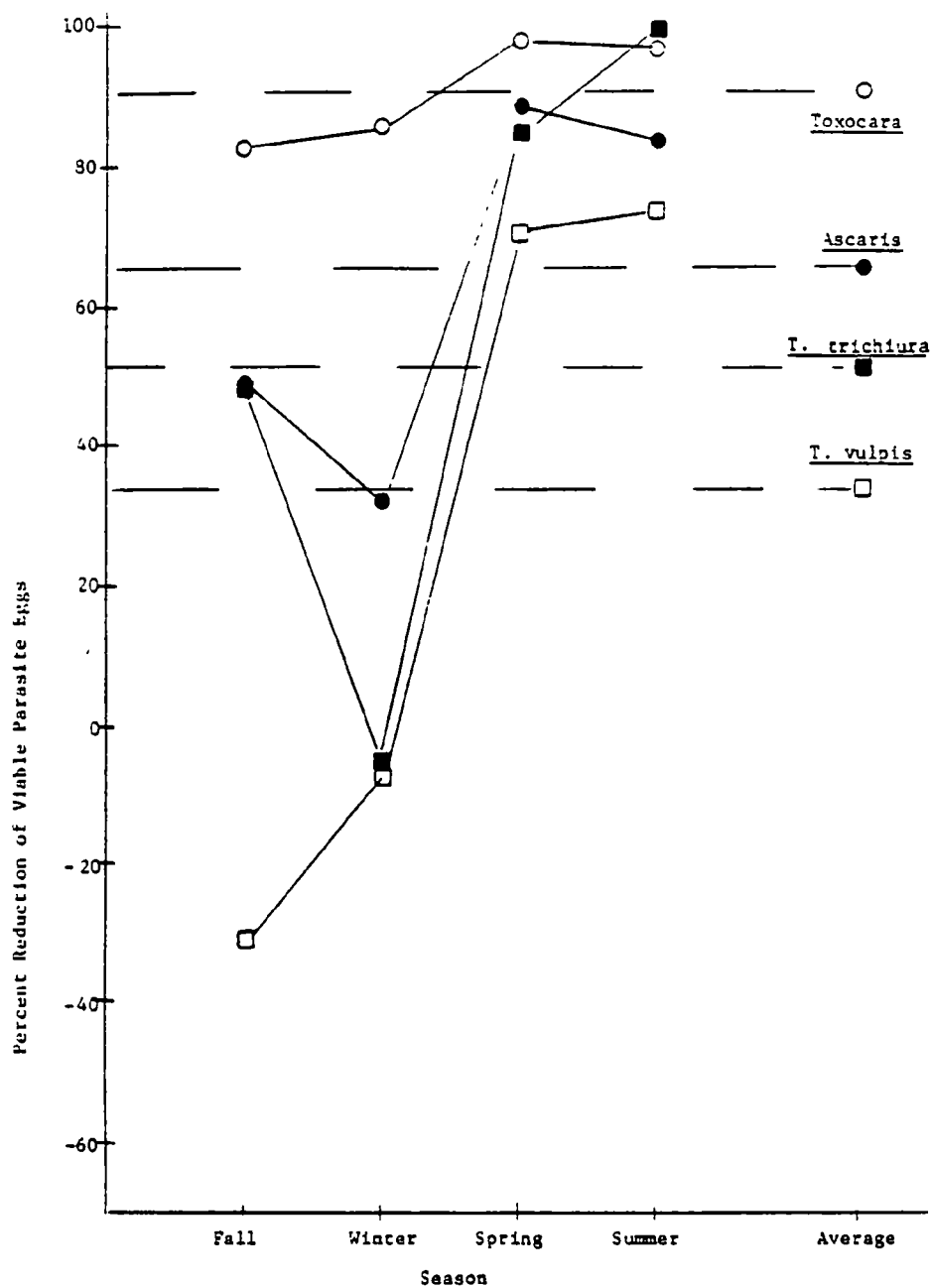


Figure 5. Percent reduction of viable parasite eggs in drying bed dewatered sludges following aerobic stabilization as compared to densities in raw sludges versus seasons and yearly averages.

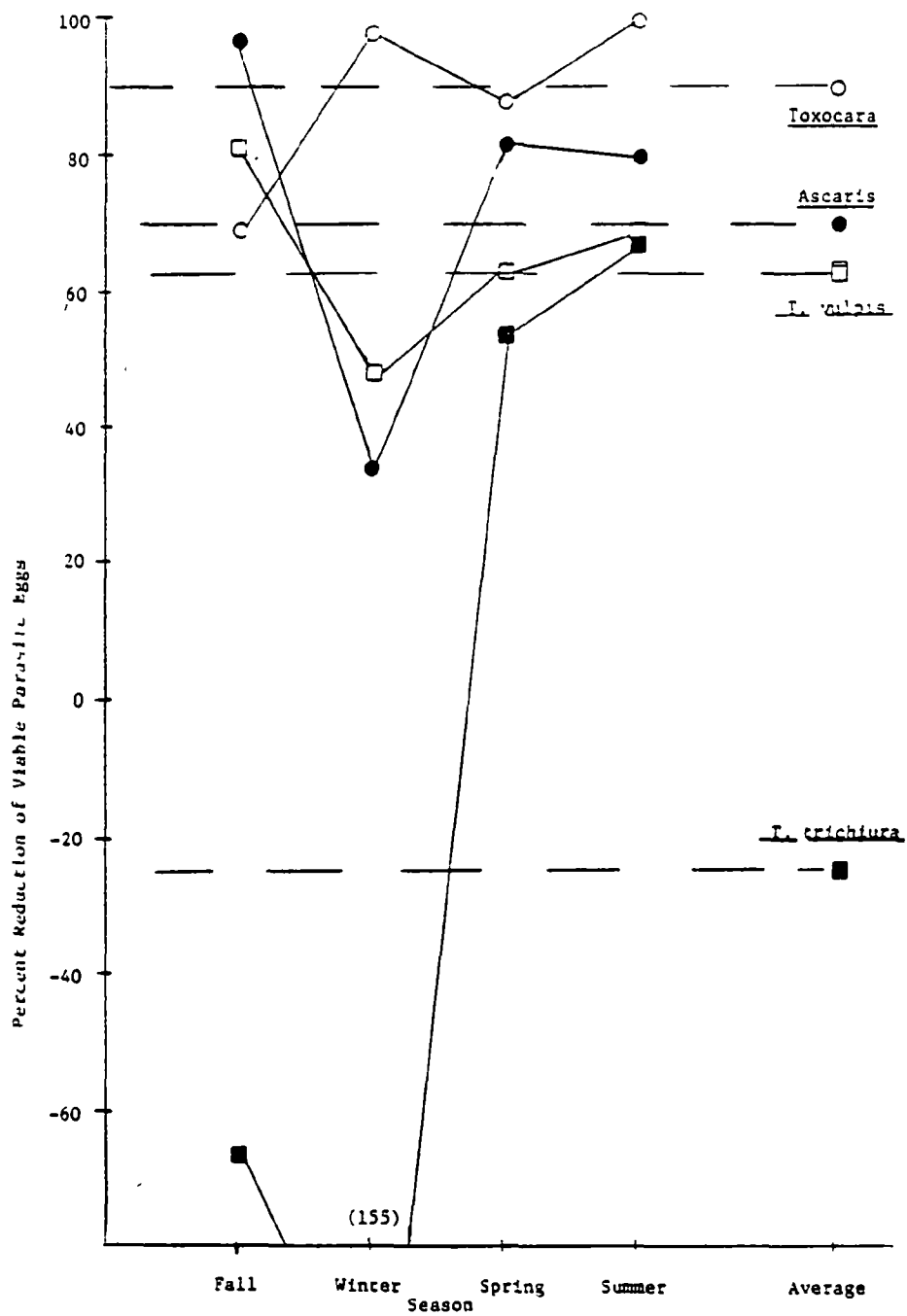


Figure 6. Percent reduction of viable parasite eggs in drying bed dewatered sludges following aerobic stabilization as compared to densities in raw sludges versus seasons and yearly averages.

et al. (91) have reviewed the literature pertaining to the resistance of Trichuris eggs to various environmental conditions. They report that ascarid eggs are more resistant to most natural conditions than are trichurid eggs, but that trichurid eggs are more resistant to ultraviolet radiation than are ascarid eggs. It is particularly interesting that several workers have reported that trichurid eggs are more susceptible to desiccation than are ascarid eggs (91).

In figures 7, 8, 9 and 10, the percent reduction of the viable eggs of Toxocara, Ascaris, T. trichiura and T. vulpis, respectively, after dewatering or sand drying beds is plotted with respect to the type of sludge stabilization process. The stabilization processes appear to have little influence on Toxocara eggs, yet Ascaris eggs seem to be influenced slightly by aerobic digestion (an increase of approximately 5 percent). Viable T. trichiura eggs were reduced over 50% following anaerobic digestion but not by aerobic digestion. The reduction of T. vulpis eggs was almost 30% greater following aerobic digestion than after anaerobic digestion (Figure 10).

Survival of Parasites in Drying Beds

In 1943, Cram (15) reported that Ascaris eggs were not completely inactivated in sludge drying beds unless the moisture content was less than five percent. Other researchers commenting on this observation (4,42) have speculated that the inactivation of Ascaris eggs noted by Cram was primarily due to desiccation. In the present study, inactivation of viable Ascaris eggs in sludge drying beds was observed at moisture concentrations well above 5 percent as shown in Figures 11 and 12. The inactivation of Ascaris in drying beds is probably due to more than desiccation alone. Factors such as temperature, oxygen content, solar radiation, exposure time, etc., may also affect survival of the eggs. In the previous section, it was also noted that the type of sludge stabilization process to which eggs have been subjected may affect subsequent survival in sludge drying beds.

The influence of moisture content of sludges in drying beds was evaluated by comparing the densities of Ascaris eggs in drying bed-sludges of different moisture contents to the densities in the raw sludge from the same plant. These field data, divided into four categories (0-20, 20-40, 40-60, and >60% moisture content), were statistically analyzed by using a multiple regression analysis. The only regression curve fit which indicated a statistically significant relationship between moisture content and Ascaris egg survival was a log-log plot relationship. The results of this analysis are shown in Table 17 and Figures 11 and 12. In general, these log-log functions were statistically significant with correlation coefficients ranging from 0.914 to 0.827 for viable Ascaris eggs and 0.768 to 0.753 for the total Ascaris eggs (viable and non-viable).

The inactivation of viable Ascaris eggs in southern drying bed sludges was observed to be over 80% at moisture contents of 60%. As shown in Figure 11, the inactivation of Ascaris eggs was observed to increase exponentially at lower moisture concentrations. The increase inactivation observed at relatively high moisture levels was probably due to a synergistic effect of

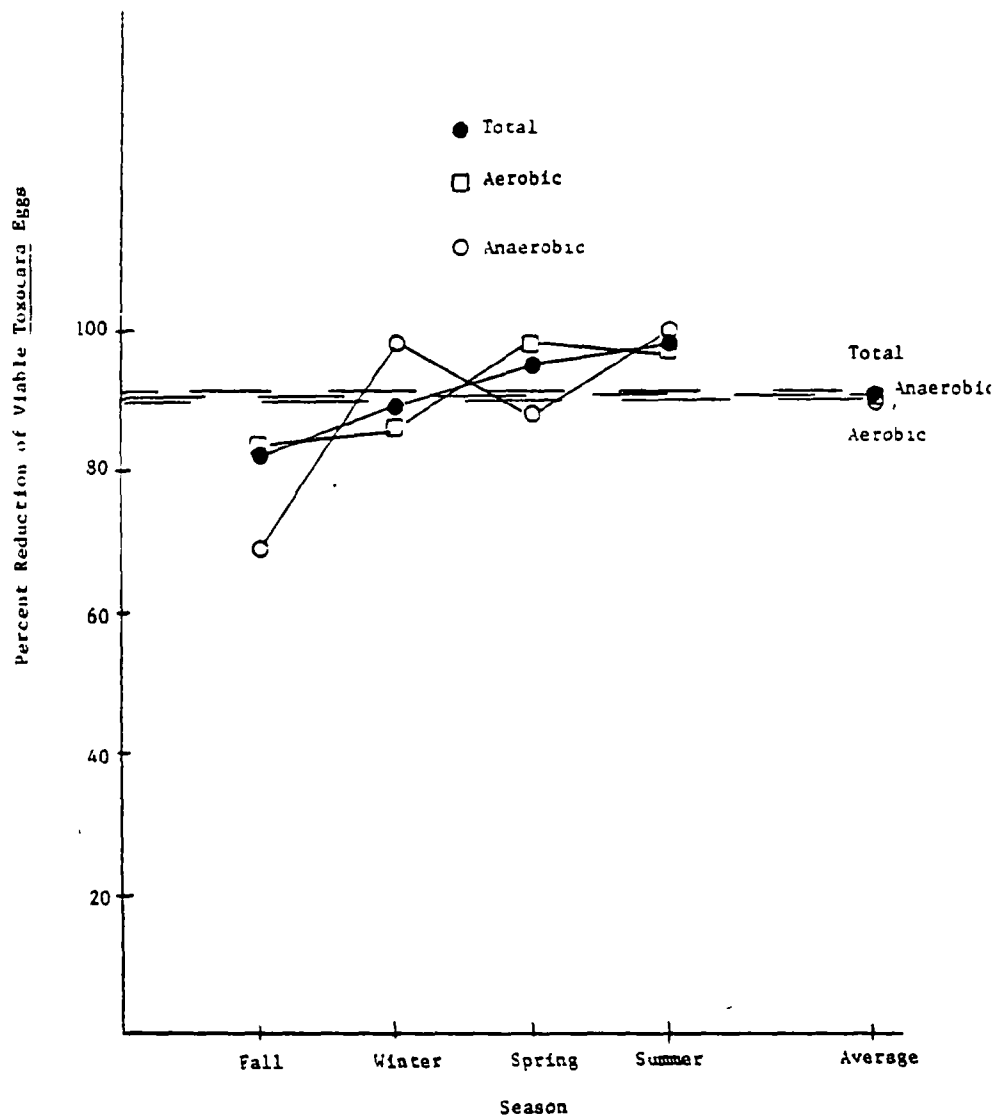


Figure 7. Percent reduction of viable *Toxocara* eggs in drying bed dewatered sludges following anaerobic, aerobic or both stabilization processes as compared to parasites in raw sludge versus season and yearly average.

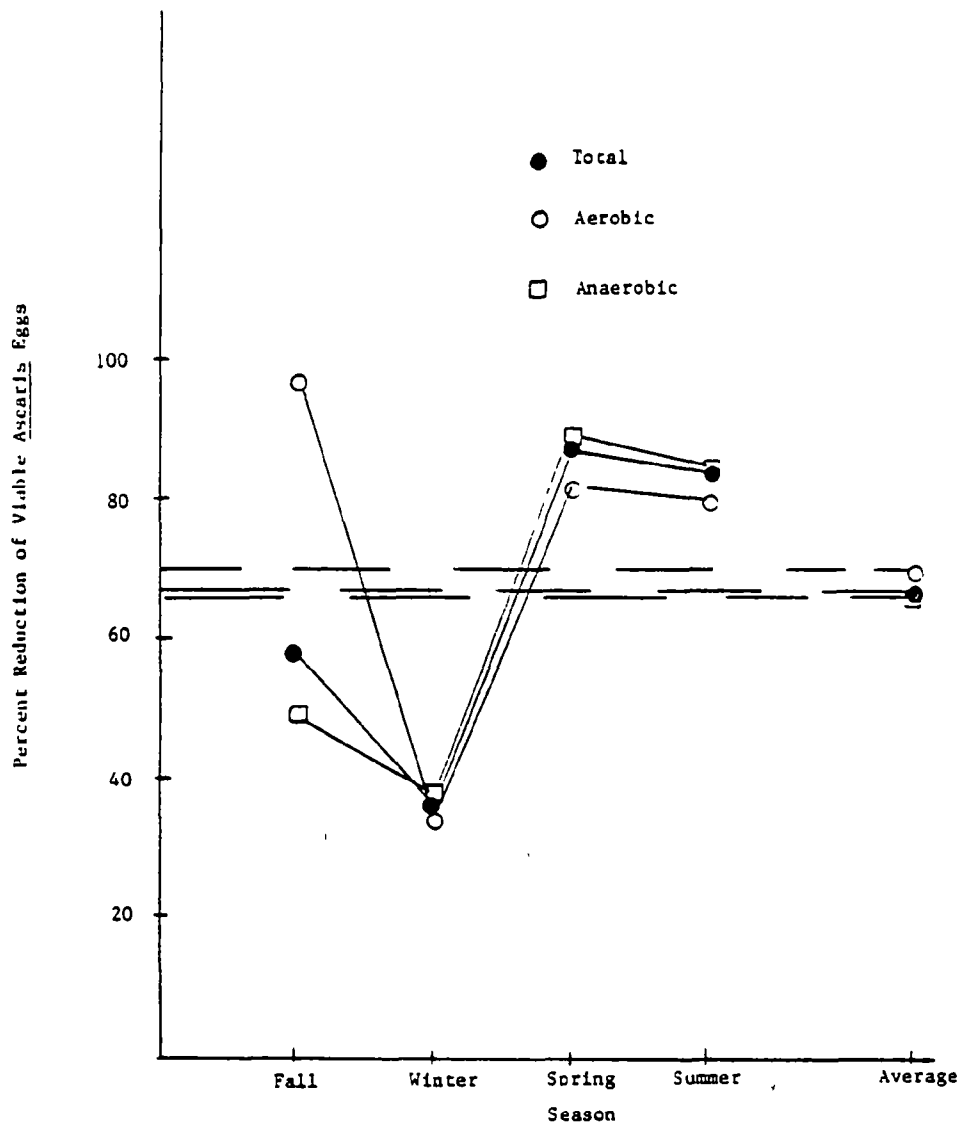


Figure 8. Percent reduction of viable *Ascaris* eggs in dewatered sludges following anaerobic, aerobic or both stabilization processes as compared to parasites in raw sludge versus season and yearly average.

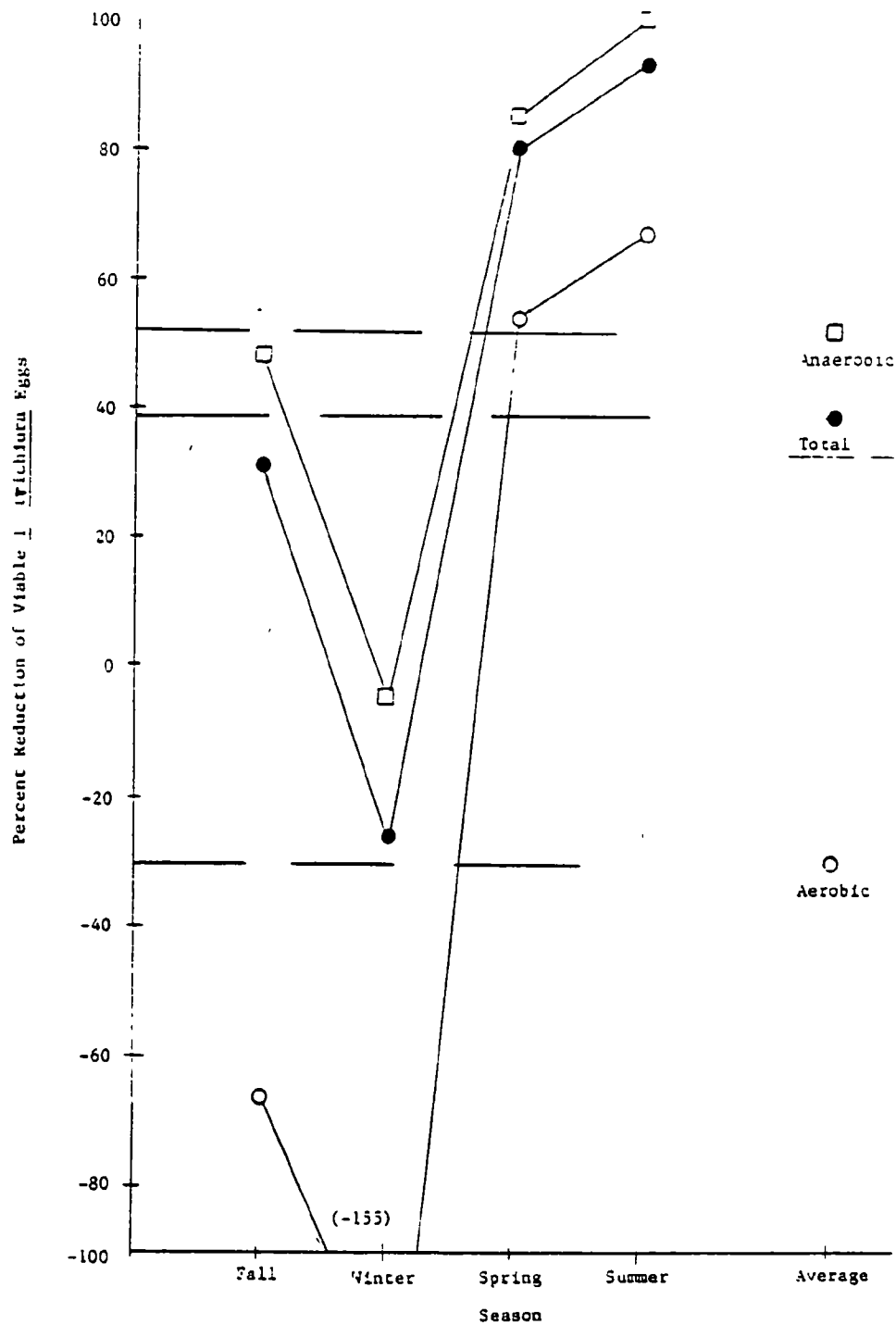


Figure 9. Percent reduction of viable *I. trichiura* eggs in drying bed dewatered sludges following anaerobic, aerobic or both stabilization processes as compared to parasites in raw sludge versus season and yearly average.

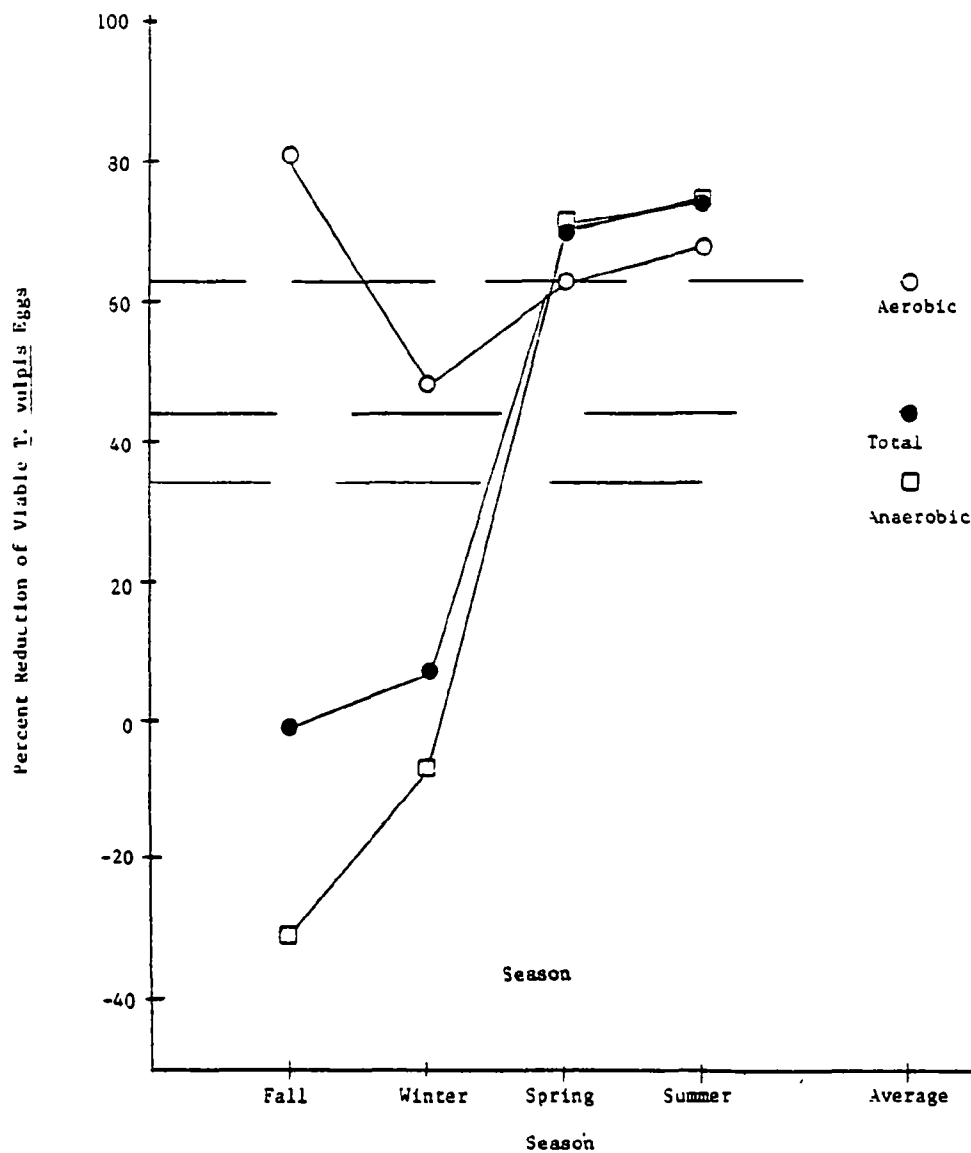


Figure 10. Percent reduction of viable *T. vulpis* eggs in drying bed dewatered sludges following anaerobic, aerobic or both stabilization processes as compared to parasites in raw sludge versus season and yearly average.

Figure 11. Plot of log of viable Ascaris egg density in drying bed versus log of viable Ascaris eggs in raw sludge with respect to moisture content

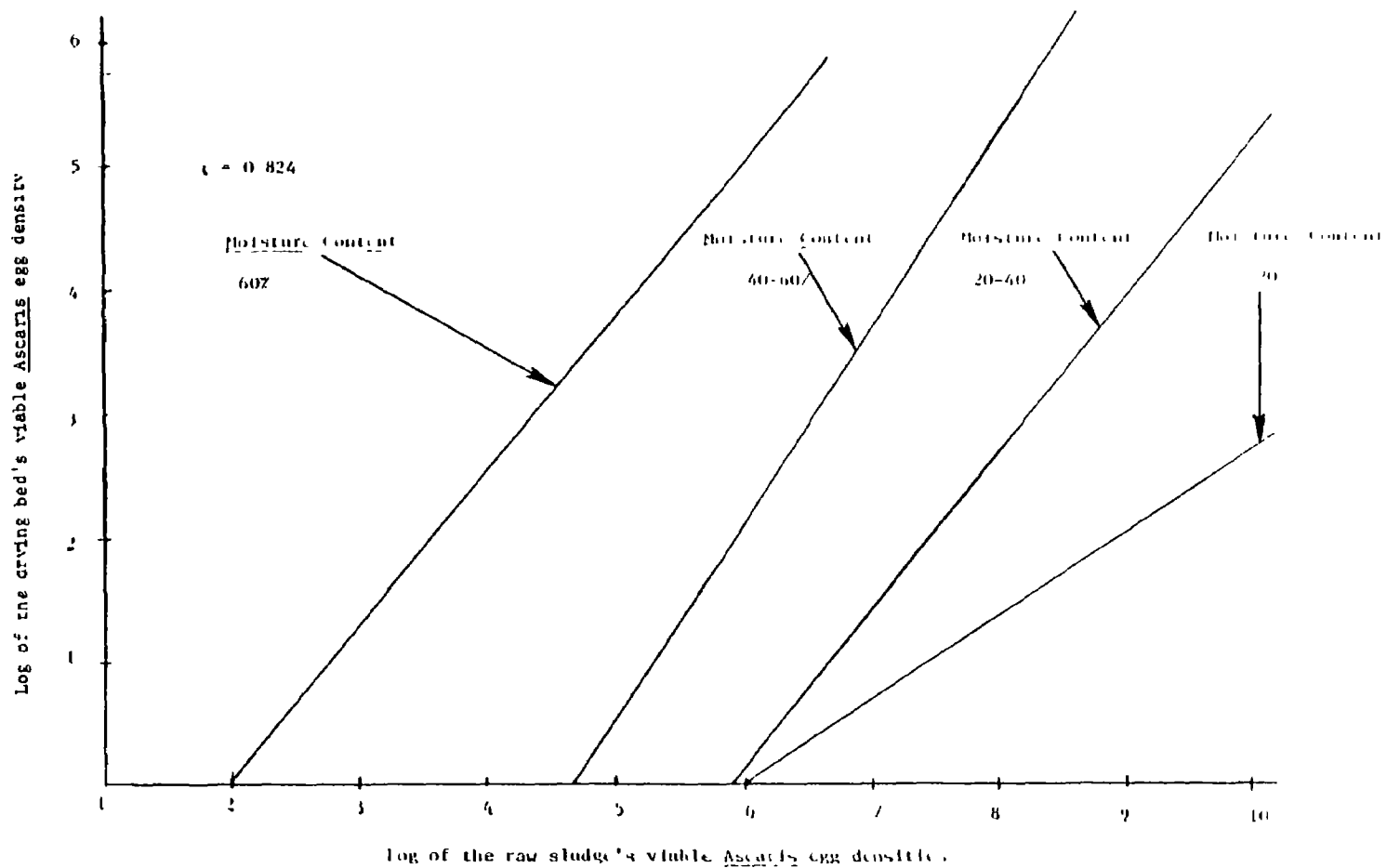
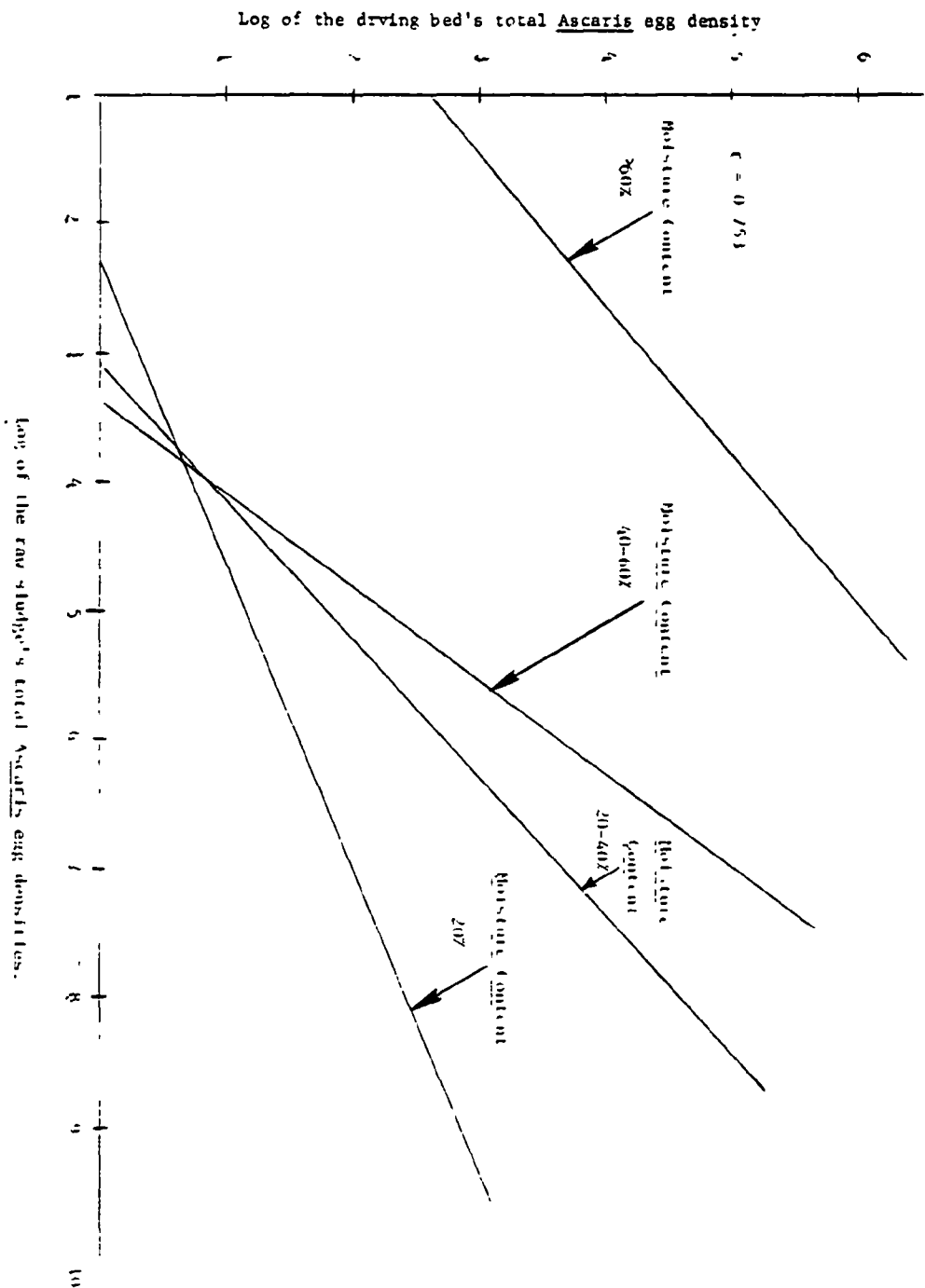


Figure 12. Plot of log of total *Ascaris* eggs in drying beds versus log of total *Ascaris* eggs in raw sludge with respect to moisture content



desiccation, temperature, solar radiation, etc. Currently, research is on-going at Tulane University to quantitate the effects of these various factors on the survival of helminth eggs in drying bed sludges.

TABLE 17. STATISTICAL DATA ON REGRESSION ANALYSIS FOR LOG-LOG PLOTS ON VIABLE AND TOTAL ASCARIS EGGS COMPARING PARASITE DENSITIES FROM THE RAW SLUDGE TO FINAL DRYING BED SLUDGES

<u>Ascaris</u> Eggs (Total or Viable)	Moisture Content (Percent)	Function (Log-Log)	Correlation Coefficients (r)
Viable	0-20	$y^{(1)} = -3.89 + 0.662x^{(2)}$	0.824
Viable	20-40	$y = -8.22 + 1.364x$	0.824
Viable	40-60	$y = -7.41 + 1.588x$	0.824
Viable	>60	$y = -2.41 + 1.24x$	0.824
Total	0-20	$y = -0.94 + 0.425x$	0.753
Total	20-40	$y = -2.79 + 0.925x$	0.753
Total	40-60	$y = -4.56 + 1.372x$	0.753
Total	>60	$y = 1.78 + 0.851x$	0.753
Viable	0-15	$y = -0.623 + 0.265x$	0.914
Viable	15-40	$y = -3.173 + 1.455x$	0.914
Viable	>40	$y = 1.047 + 0.670x$	0.914
Total	0-15	$y = 1.87 - 0.394x$	0.768
Total	15-40	$y = -1.68 + 1.046x$	0.768
Total	>40	$y = -0.98 + 1.176x$	0.768

(1)y = Log at parasite egg density in drying bed (#eggs/kg dry wt.).

(2)x = Log at parasite egg density in raw sludge (#eggs/kg dry wt.).

The densities of total Ascaris eggs were found to decrease substantially when the moisture content of the drying bed sludges was less than 60% (Figure 12). As the moisture content decreased the total number of viable and non-viable Ascaris eggs also decreased, but the reduction was not exponential as was noted with only viable Ascaris eggs.

The relationship between viable eggs as a percentage of total eggs of Ascaris and Toxocara and moisture content in drying beds is shown in Figure 13. Ascaris eggs were found to be more resistant than Toxocara eggs to the effects of decreasing moisture contents. The 100 percent inactivation of Ascaris eggs was graphically estimated to be at approximately 23% sludge moisture content, and for Toxocara eggs at a moisture content of about 35%. The correlation coefficients were 0.71 and 0.54 for viable Ascaris and Toxocara eggs, respectively. Because of these low correlation coefficients and the wide scatter in data points at low moisture contents, these estimates should be taken as an indication of only general trends.

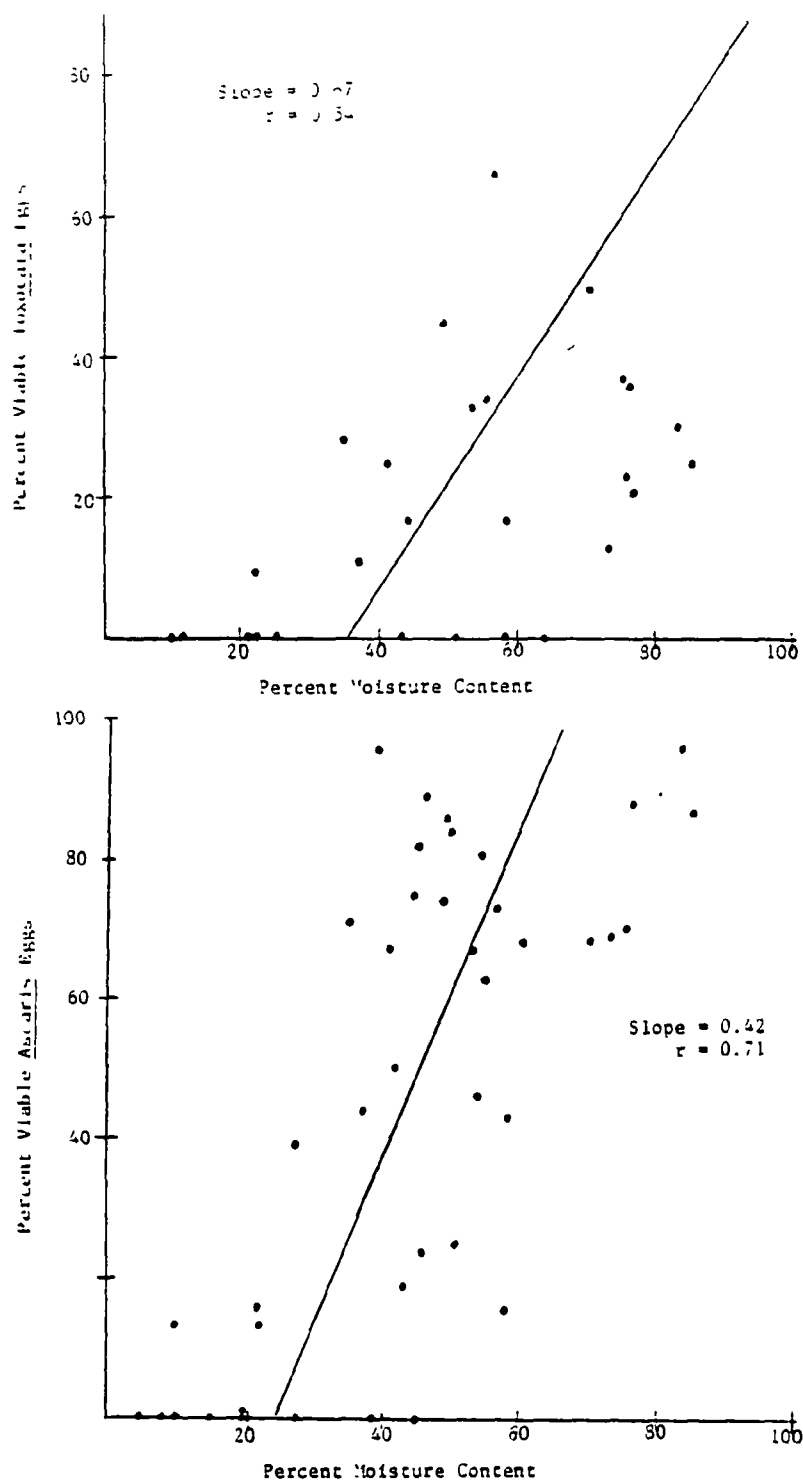


Figure 13. Percentage of viable Toxocara and Ascaris eggs versus percentage of moisture content.

The viability of Ascaris and Toxocara eggs in drying bed sludges as related to moisture content was also analyzed for each season of the year. Table 18 lists for each season the moisture content of sludge drying bed samples in which no viable Ascaris or Toxocara eggs were observed. The moisture concentrations where the total Ascaris or Toxocara eggs were inactivated were found to be 5 percent in the fall, 7 percent in the winter, 8 percent in the spring, and 15 percent in the summer. Only during the summer did there appear to be any substantial change. The number of drying bed samples in which both Ascaris and Toxocara eggs were found non-viable followed an expected trend in regard to season; i.e., 7 samples in the fall, 4 samples in the winter, 12 samples in the spring, and 14 samples in the summer. In Figures 14 and 15, the percentages of viable Ascaris and Toxocara eggs, respectively, recovered from drying bed sludges are plotted against moisture content by season. These plots were not found statistically significant and it is probable that many other factors influence the inactivation of these eggs.

TABLE 18. THE INFLUENCE OF DRYING BEDS TO COMPLETELEY INACTIVATE ASCARIS, TOXOCARA, OR BOTH EGGS WITH RESPECT TO SEASON AND MOISTURE CONTENTS.

SEASON	NUMBER OF DRYING BED SAMPLES ANALYZED	NUMBER OF SAMPLES WITH NO VIABLE EGGS	NUMBER OF SAMPLES WITH VIABLE EGGS ABOVE MINIMUM MOISTURE CONTENTS	LOWEST MOISTURE CONTENTS BELOW WHICH NO VIABLE EGGS OBSERVED	NUMBER OF SAMPLES WITH NO VIABLE EGGS AT OR BELOW LOWEST MOISTURE CONTENTS
For Both <u>Ascaris</u> and <u>Toxocara</u> Eggs					
Fall	24	7	17	5%	1
Winter	22	4	18	7%	1
Spring	22	12	10	8%	8
Summer	21	14	7	15%	8
<u>Ascaris Eggs Only</u>					
Fall	21	7	14	5%	1
Winter	22	5	17	7%	1
Spring	22	12	10	8%	8
Summer	21	14	7	15%	8
<u>Toxocara Eggs Only</u>					
Fall	24	12	12	5%	1
Winter	22	12	10	21%	3
Spring	22	17	5	21%	11
Summer	21	18	3	20%	10

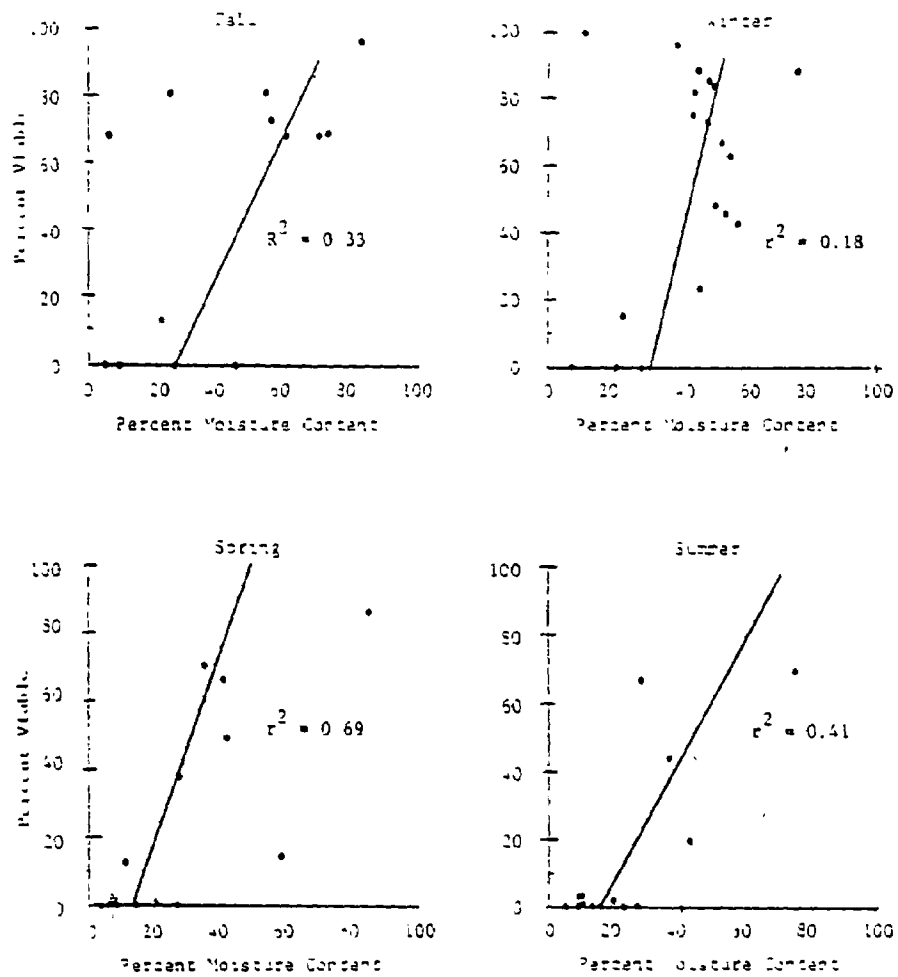


Figure 14. Percentage of viable *Ascaris* eggs versus percentage moisture content for each season in drying beds

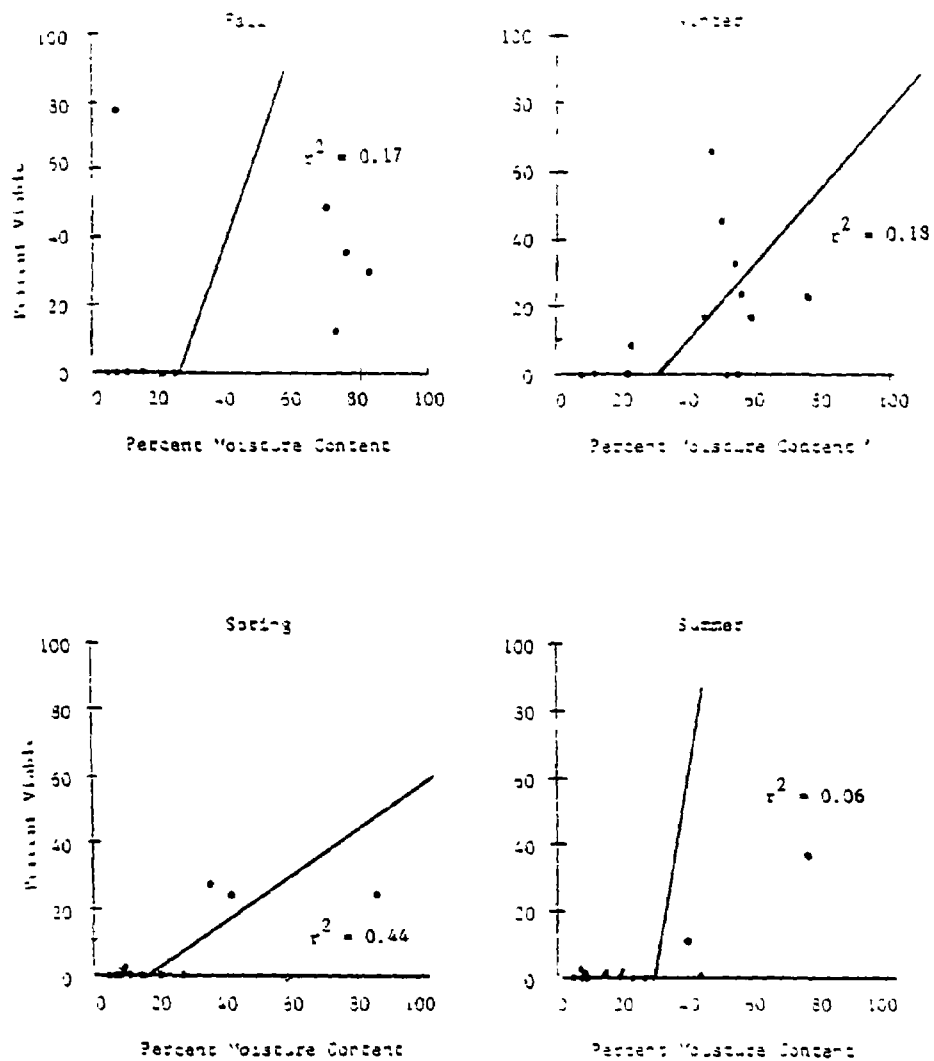


Figure 15. Percentage of viable *Toxocara* eggs versus percentage moisture content for each season in driving beds

Mass Balance of Ascaris Eggs Through a Secondary Wastewater Treatment System

One municipal wastewater treatment plant which had high densities of parasites in the influent raw wastewater due to the contribution of abattoir wastewater was evaluated for a mass balance of parasite eggs through each of its major unit processes. Unit operations at the plant consisted of contact stabilization, aerobic sludge stabilization and sludge drying beds as shown in Figure 16. Concentrations of Ascaris eggs were highest prior to peak flows during early morning and late afternoon; whereas general municipal constituents fluctuated as expected (Table 19). A possible explanation of this phenomena is that the Ascaris eggs originating from the abattoir discharge settled in the sewage lines until high flows caused suspension and conveyance to the treatment plant.

The secondary clarifier was observed to remove 91 to 98% of the Ascaris eggs in the contact stabilized effluent. As shown in Table 20, 5 to 51 viable Ascaris eggs per liter of effluent were discharged into a receiving creek. The percent removal of parasite eggs varied with clarifier overflow rate as illustrated in Figure 17. As the overflow rate increases through the clarifier, the parasite eggs are scourged along with light solids over the weir into the final effluent.

The Ascaris eggs removed by secondary clarification are recycled with the influent sewage in the return sludge, thus effectively concentrating the eggs in the contact stabilized and reaerated sludge. Therefore, it would appear that treatment plants utilizing contact stabilization, activated sludge, or extended aeration, tend to equilibrate the parasite eggs in the sludges of the treatment plant.

LABORATORY STUDIES

Introduction

The literature and recent research studies indicate that several domestic waste treatment processes are capable of either removing or inactivating eggs of parasites as shown in Table 3. The effectiveness of these processes varies considerably depending upon the type of waste, climate, parasite, etc.

Laboratory studies on select wastewater sludges were conducted to determine factors involved in the inactivation or the inhibition of parasite eggs and cysts in sewage sludge. Bench scale results of continuous aerobic and anaerobic sludge digestion, lime stabilization, ammonification, sonication, and combinations of the above processes for the destruction of Ascaris suum and Toxocara canis eggs in sludges are discussed in the following:

Aerobic Digestion

The aerobic digestion experiments were conducted at temperatures of 28°C, 35°C, 45°C, and 55°C at a ten day hydraulic retention time. After the

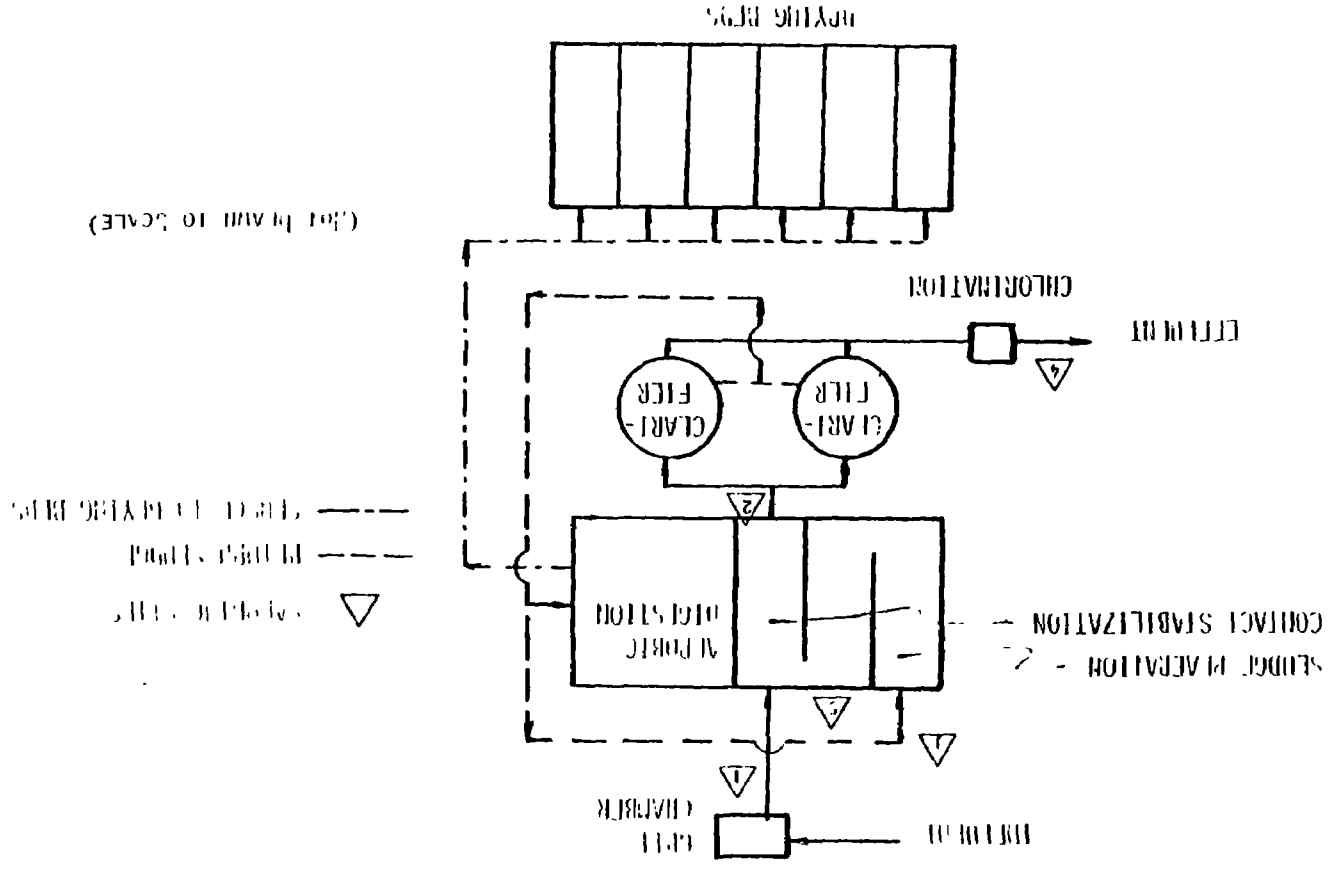


TABLE 19. WASTE CHARACTERISTICS OF INFLUENT RAW WASTEWATER
FOR PLANT RECEIVING ABATTOIR WASTE

Ascaris Content (#Viable eggs/l)	Chemical Oxygen Demand (mg/l)	Suspended Solids (mg/l)	Flow (MGD)	Time (Hour)
257	416	550	0.9	0800
4	-	350	2.0	1400
29	904	700	2.6	2000
41	248	100	1.0	0200
2,933	64	100	1.6	0800
11	400	50	2.2	1400
176	784	400	2.4	2000
7	672	300	2.5	0200

TABLE 20. EFFECTIVENESS OF SECONDARY CLARIFICATION TO REMOVE
ASCARIS EGGS FROM WASTEWATER FOR MASS BALANCE STUDY

Influent ¹ (# Viable <u>Ascaris</u> Eggs/liter)	Effluent (# Viable <u>Ascaris</u> Eggs/liter)	Removal (%)	Flow (MCD)
195	11	94.4	2.4
182	8	95.6	2.4
576	51	91.1	2.3
503	- ²	-	0.7
207	5	97.6	1.8
331	11	96.7	2.2
299	11	96.3	2.7
1,041	18	98.2	0.8

1 Influent from contact stabilization process.

2 Sample lost.

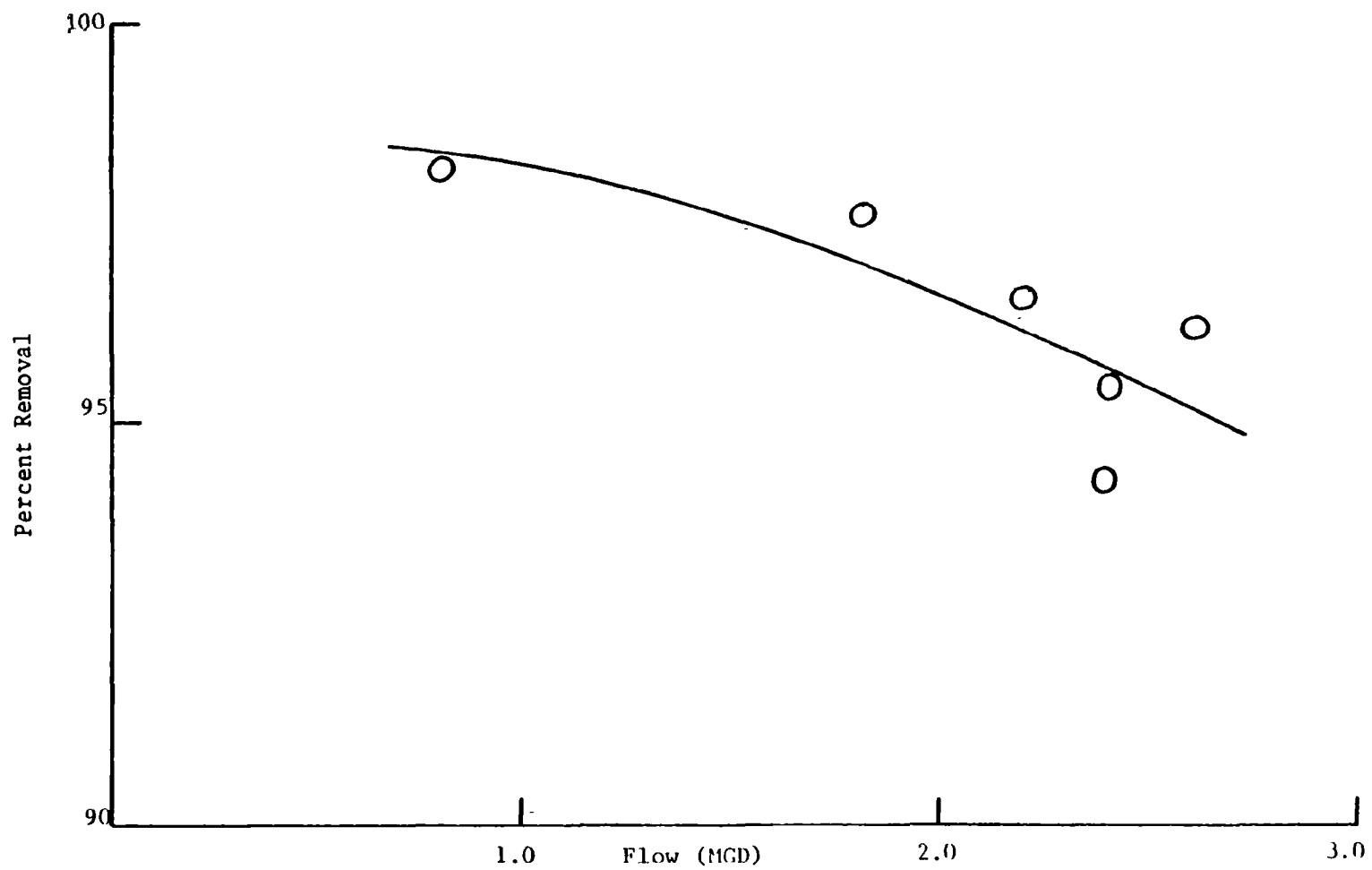


Figure 17. Percent removal of viable Ascaris eggs with respect to overflow rate by the secondary clarifier.

digesters had attained a steady condition (within two months), known numbers of Ascaris and Toxocara eggs were added, at one time to the aerobic digesters and at density levels that would be adequate for subsequent analysis. Raw secondary sludge (2 liters) was added on a batch basis every other day after 2 liters of sludge was removed from the digester. Each sludge sample was analyzed for density and viability of Ascaris and Toxocara eggs. Since the Ascaris and Toxocara eggs were spiked into the digester only once, the total number of eggs in the digesters and the number of eggs per unit volume decreased after each sampling.

The problems related to the use of a reliable indicator organism were discussed in the research methodology section. In the initial aerobic digestion experiments, eggs of Ascaris suum obtained from the uteri of worms were used, but eggs obtained from the intestinal contents of swine were found to be more reliable and were used in subsequent experiments. Figures 18, 19, and 20 indicate that the results obtained in experiments using the two types of eggs are quite different. In experiments using uterus-derived eggs (Figure 18), the recovery of eggs was significantly reduced after two days at 35°C. However, when eggs from the intestinal contents of swine were used in experiments at 35°C, little or no reduction in their recovery was noted over a period of ten days (Figure 20). These results may be due to differences in the environment in the aerobic digesters used in the two experiments, to differences in the resistance of uterus-derived eggs and eggs from swine intestines, or to a problem in recovering uterus-derived eggs by the technique used for parasite analysis.

As indicated by the literature, temperature has a considerable influence on the survival of Ascaris and Toxocara eggs. Figure 20 indicates that Ascaris eggs from the intestinal contents of swine are not affected by temperatures up to 35°C, but at 45°C or 55°C, there appears to be an effective die-off within two days or less. At 35°C, the density of viable Toxocara eggs decreased 92.1 percent (standard deviation = 7.56), while at 45°C, which was effective in killing Ascaris eggs, the reduction of viable Toxocara eggs was only 68.4 percent (standard deviation = 10.85).

Inactivation of Ascaris and Toxocara eggs in the aerobic digesters occurred mostly during the first two days. There was little, if any, additional inactivation observed after this time.

As noted in Figures 18, 19 and 20, aerobic digestion in continuous digesters (retention time 10 days) at ambient temperatures appeared to be ineffective in reducing the number of viable eggs of Ascaris. During the batch digestion experiment, however, a 96 percent reduction in viable Ascaris eggs was observed after 20 days (69 percent after 10 days) as shown in Table 21. An explanation for this difference is that an increased digestion time results in a reduction of the carbon source which in turn will alter the microbial population.

Anaerobic Digestion

The anaerobic digestion experiments were conducted at temperatures of 35°C, 45°C, and 55°C with a fifteen day hydraulic retention time. The anaero-

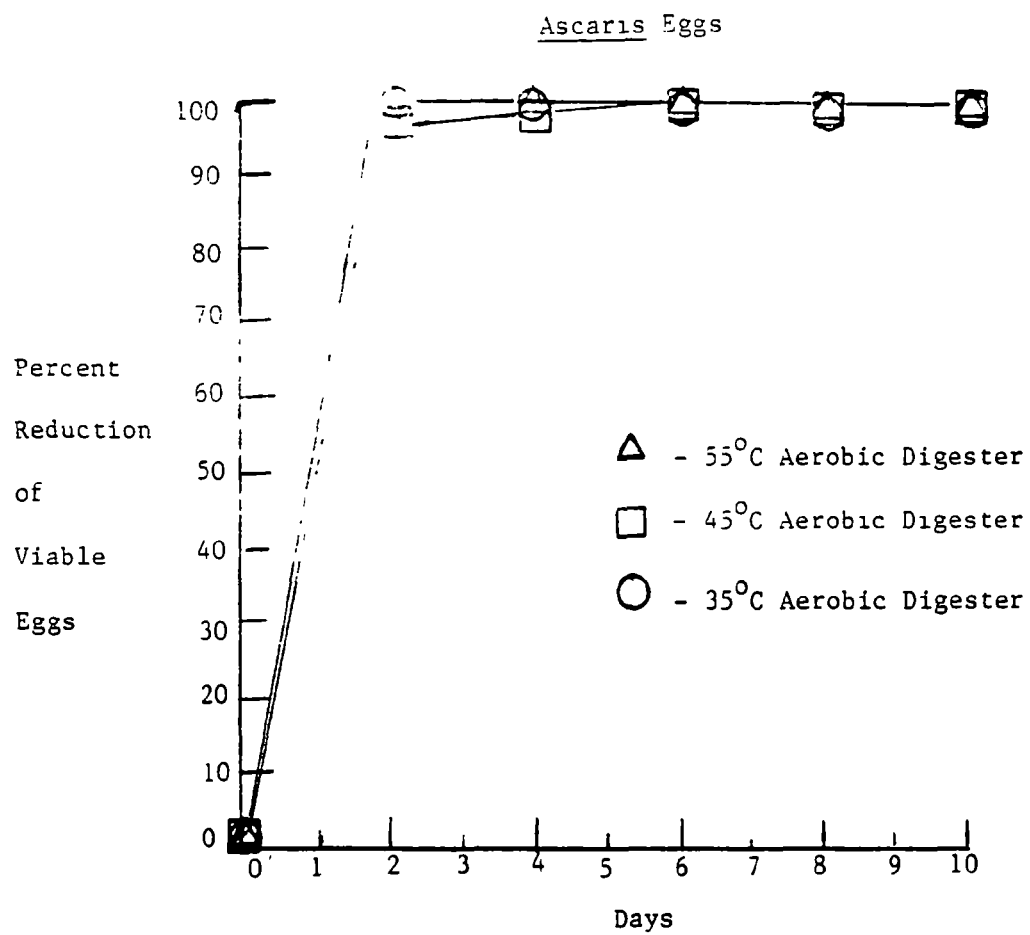


Figure 18. Effects of aerobic digestion on Ascaris eggs (from the uteri of worms).

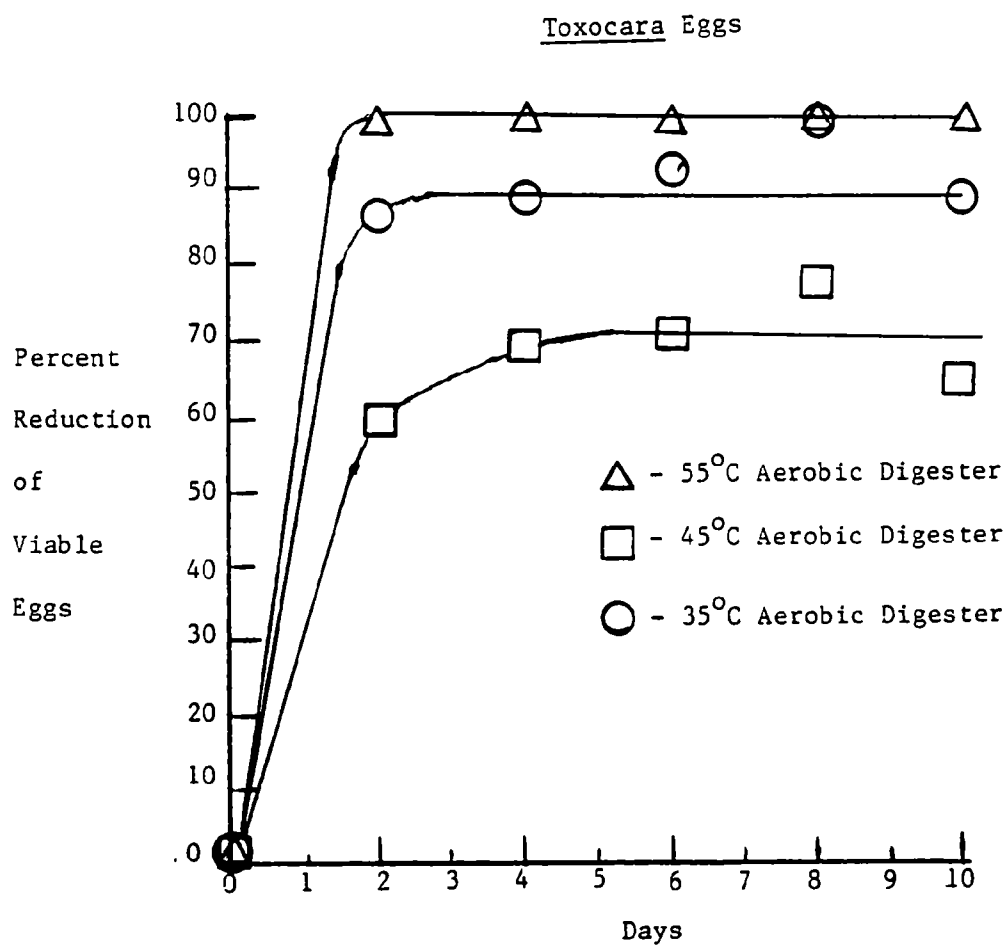


Figure 19. Effects of aerobic digestion on Toxocara eggs (from dog feces).

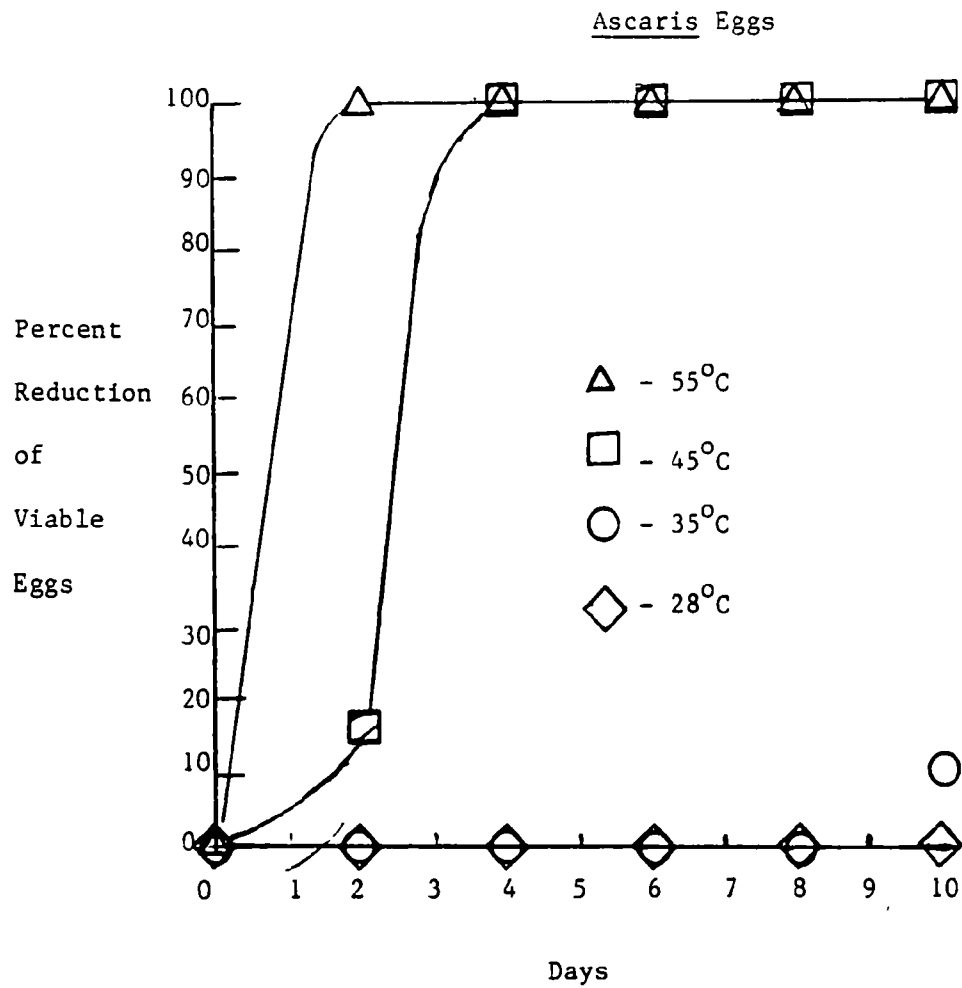


Figure 20. Effects of aerobic digestion on Ascaris eggs (from the small intestinal contents of swine).

bic digesters required three months to obtain a steady state or equilibrium condition. After this period, known quantities of Ascaris and Toxocara eggs were added at one time so that the parasite densities in the sludge would be adequate for parasite analysis. Raw secondary sludge (1½ liters) was added every two days after 1½ liters of digested sludge had been removed. After the sludge had been spiked with parasites, samples of digested sludge were removed after 20 minutes, 2 days, 6 days, 10 days and 15 days and were analyzed for the density and viability of the Ascaris and Toxocara eggs. Since the Ascaris and Toxocara eggs were added only once, the total number of eggs in the digester and the number per unit volume decreased after each sampling.

As shown in Figure 21, anaerobic digestion had similar effects on Ascaris and Toxocara eggs. At 35°C, the viability of Ascaris and Toxocara eggs decreased linearly by approximately 30 to 40 percent over a 15 day period; yet at 45°C, the viability of Ascaris and Toxocara eggs decreased to 60 and 100 percent, respectively, within 20 minutes, with complete inactivation of Ascaris eggs occurring within two days (Table 22). At 55°C, both Ascaris and Toxocara eggs were inactivated within 20 minutes.

TABLE 21. BATCH AEROBIC DIGESTION OF RAW PRIMARY
SLUDGE SPIKED WITH ASCARIS EGGS (AT 28°)

Time	Number of Viable <u>Ascaris</u> Eggs/50 ml	Percent Reduction of Viable <u>Ascaris</u> Eggs
1 hour	48	-
5 days	51	0
10 days	15	69
15 days	10	79
20 days	2	96

TABLE 22. INFLUENCE OF ANAEROBIC DIGESTION AT 35°C, 45°C,
AND 55°C ON THE VIABILITY OF ASCARIS AND TOXOCARA EGGS IN
RAW SECONDARY SLUDGE OVER A FIFTEEN-DAY PERIOD

Time	Percent Non-Viable					
	<u>Ascaris</u> Eggs			<u>Toxocara</u> Eggs		
	35°C	45°C	55°C	35°C	45°C	55°C
20 min.	0	60	100	0	100	100
2 days	5	100	100	0	100	100
6 days	30	100	100	18	100	100

(continued)

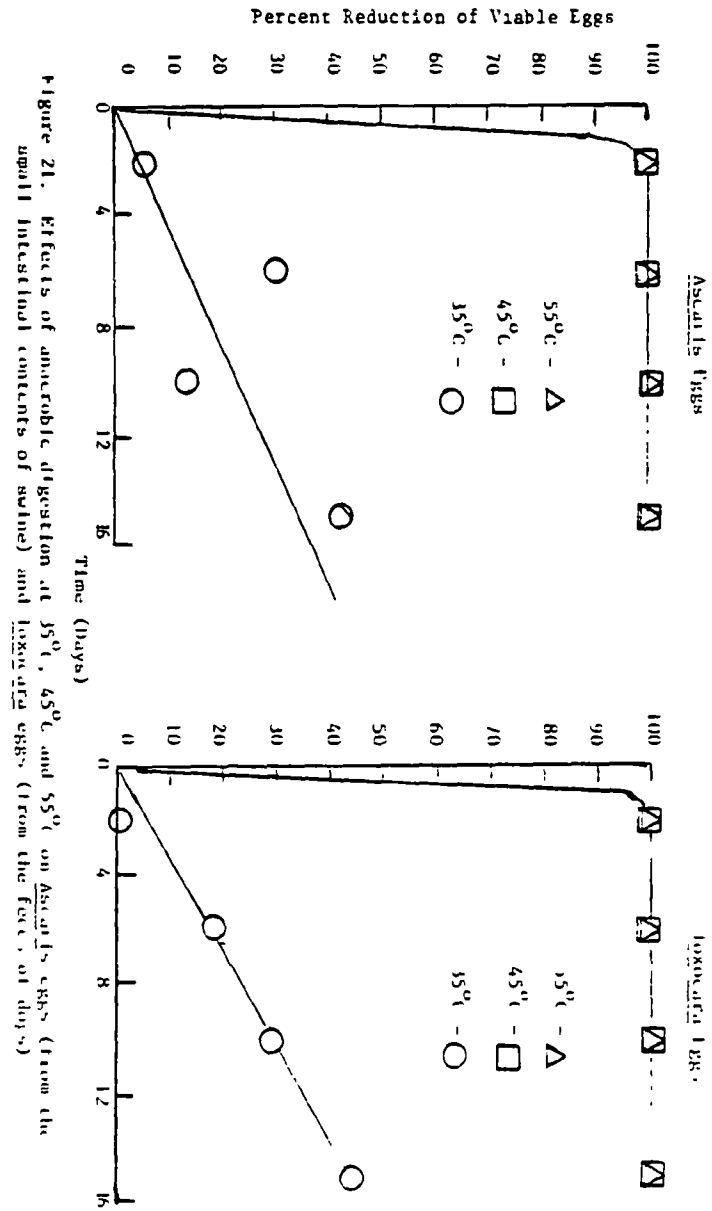


Figure 21. Effects of anaerobic digestion at 35°C, 45°C and 55°C on *Ascaris* eggs (from the small intestinal contents of swine) and *Toxocara* eggs (from the feces of dogs).

TABLE 22. (continued)

Time	Percent Non-Viable					
	<u>Ascaris</u> Eggs			<u>Toxocara</u> Eggs		
	35°C	45°C	55°C	35°C	45°C	55°C
10 days	13	100	100	29	100	100
15 days	42	100	100	34	100	100

Lime Stabilization

Since lime stabilization has been successful in the inactivation of bacterial and viral pathogens, the potential of lime stabilization for destroying parasites was investigated. Raw primary sludge, sludge aerobically-digested at 28°C and sludge aerobically-digested at 35°C were used in these laboratory studies. Lime was added to each type of sludge at dosages of 0, 100, 1000, and 3000 milligrams of calcium hydroxide per gram of suspended solids and samples of these sludges were then maintained under aerobic and anerobic conditions for 20 days.

The effects of lime stabilization on Ascaris eggs in raw primary sludge maintained under aerobic conditions at ambient temperature (about 28°C) are shown in Table 23 and Figure 22. In this experiment in which the Ascaris eggs used were from the intestinal contents of swine, the recovery of viable eggs decreased 80% in 5 days and 100% in 10 days when 1000 mg lime/gm dried sludge solids were added to raw primary sludge under aerobic conditions, and 92% in 5 days and 100% in 10 days when 3000 mg lime/g dried sludge solids were added (Table 23). No significant effect was noted when 100 mg lime/g dried sludge solids were added.

The effects of lime stabilization on aerobically digested sludges at 28 and 35°C were examined under both aerobic and anaerobic conditions at ambient temperature (28°C) for 20 days. The results of the studies are shown in Table 24. In the 28°C aerobically-digested sludge, as shown in Figure 23, the viability of Ascaris eggs was reduced 100% within 20 days under anaerobic conditions when 100 mg of lime per gram of dry sludge solids was added. Under aerobic conditions with the same lime dosage there was no reduction in the viability of Ascaris eggs. This difference at the low lime dosages (100 mg of lime or less) is probably due to the increased bioactivity in the aerobic environment. At higher dosages (1000 mg or greater), the influence of lime on the viability of Ascaris eggs was more noticeable under aerobic conditions within the first hour.

TABLE 23. EFFECTS OF LIME STABILIZATION ON ASCARIS EGGS FROM THE INTESTINAL CONTENTS OF SWINE IN PRIMARY SLUDGE UNDER AEROBIC CONDITIONS.

Time	% Reduction of Viable <u>Ascaris</u> Eggs ¹
<u>No Lime Dosage</u>	
1 hour	-
5 days	0
10 days	69
15 days	79
20 days	96
<u>100 mg of Lime (Ca(OH)₂) Per Gram Susp. Solids</u>	
1 hour	-
5 days	11
10 days	51
15 days	-
20 days	-
<u>1000 mg of Lime (Ca(OH)₂) Per Gram Susp. Solids</u>	
1 hour	-
5 days	80
10 days	100
15 days	100
20 days	100
<u>3000 mg of Lime Ca(OH)₂) Per Gram Susp. Solids</u>	
1 hour	-
5 days	92
10 days	100
15 days	100
20 days	100

¹Percent Reduction = Percent of viable eggs noted to be reduced from the number of viable eggs found after one hour operation.

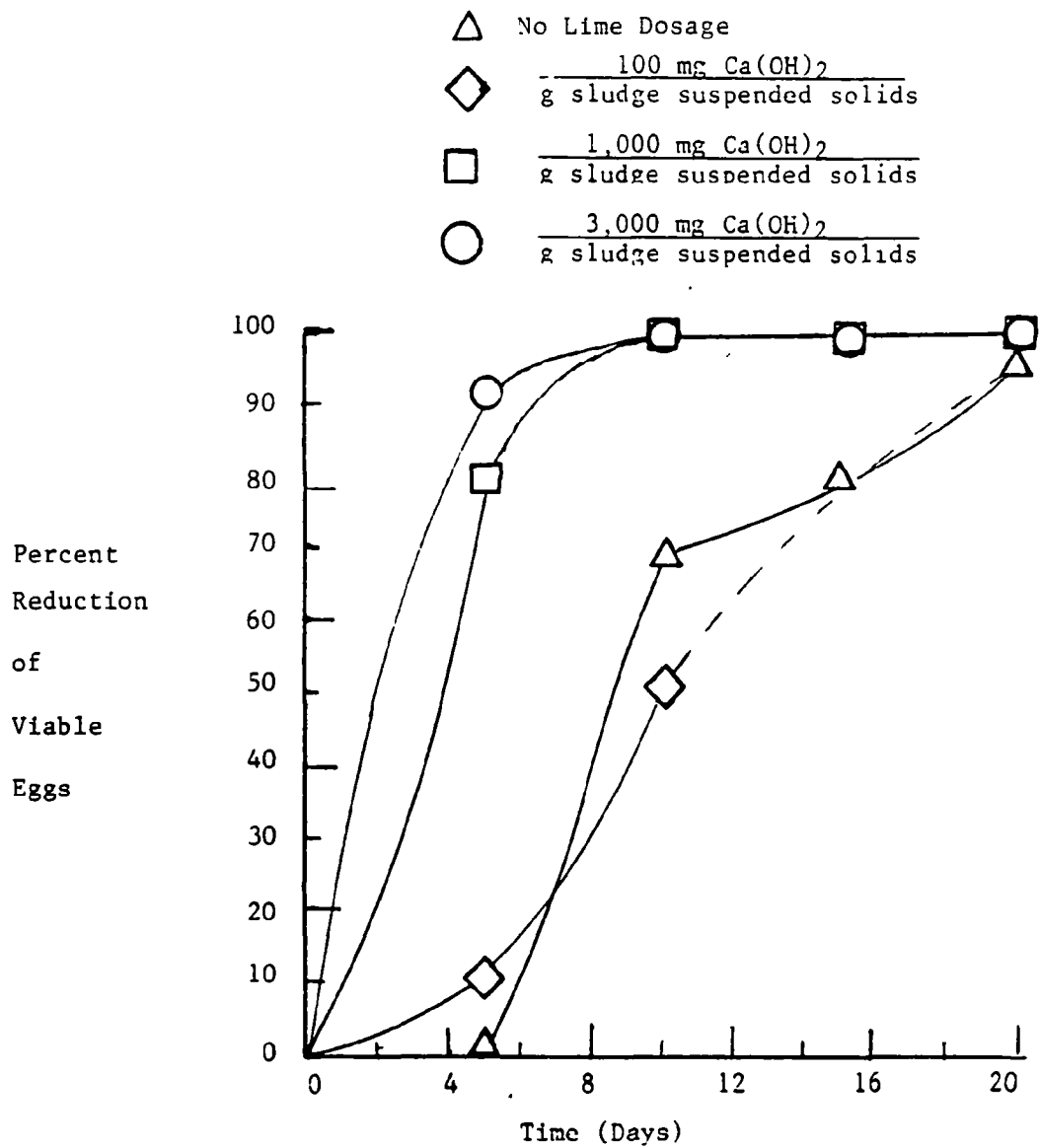


Figure 22. Effect of lime stabilization on Ascaris eggs (from the small intestines). Raw primary sludge was limed, and then maintained under aerobic conditions at ambient temperature.

TABLE 24. EFFECTS OF LIME STABILIZATION ON ASCARIS EGGS (FROM INTESTINAL CONTENTS OF SWINE) ADDED TO SLUDGES (AEROBICALLY DIGESTED AT 28°C OR 35°C) AND THEN MAINTAINED AT AMBIENT TEMPERATURES UNDER EITHER ANAEROBIC OR AEROBIC CONDITIONS AFTER LIME ADDITION
(DATA PRESENTED IN PERCENT REDUCTION IN VIABLE ASCARIS EGGS.)¹

<u>Time</u>	<u>28°C Aerobically Digested Sludge Limed</u>		<u>35°C Aerobically Digested Sludge Limed</u>	
	<u>Aerobic Conditions</u>	<u>Anaerobic Conditions</u>	<u>Aerobic Conditions</u>	<u>Anaerobic Conditions</u>
<u>No Lime Dosage</u> ²				
1 hour	-	-	-	-
5 days	61	26	38	39
10 days	0	0	0	0
15 days	0	0	41	29
20 days	42	0	28	6
<u>100 mg of Lime (Ca(OH)₂) Per Gram Susp. Solids</u>				
1 hour	-	-	-	-
5 days	0	52	44	0
10 days	0	86	8	0
15 days	0	0	0	0
20 days	0	100	77	0
<u>1000 mg of Lime (Ca(OH)₂) Per Gram Susp. Solids</u> ³				
1 hour	89	-	100	-
5 days	93	100	100	26
10 days	71	60	100	37
15 days	0	0	92	0
20 days	100	87	98	0
<u>3000 mg of Lime (Ca(OH)₂) Per Gram Susp. Solids</u> ³				
1 hour	100	-	98	-
5 days	100	96	100	93
10 days	100	93	100	98
15 days	100	32	100	9
20 days	100	93	100	0

¹ Ascaris eggs were added to primary sludge prior to aerobic digestion at 28 °C or 35 °C.

² Percent reduction = Percent of viable eggs noted to be reduced from the number of viable eggs found at one hour operation.

³ Percent Reduction = Percent of viable eggs noted to be reduced from the one hour reading from the non-limed sample.

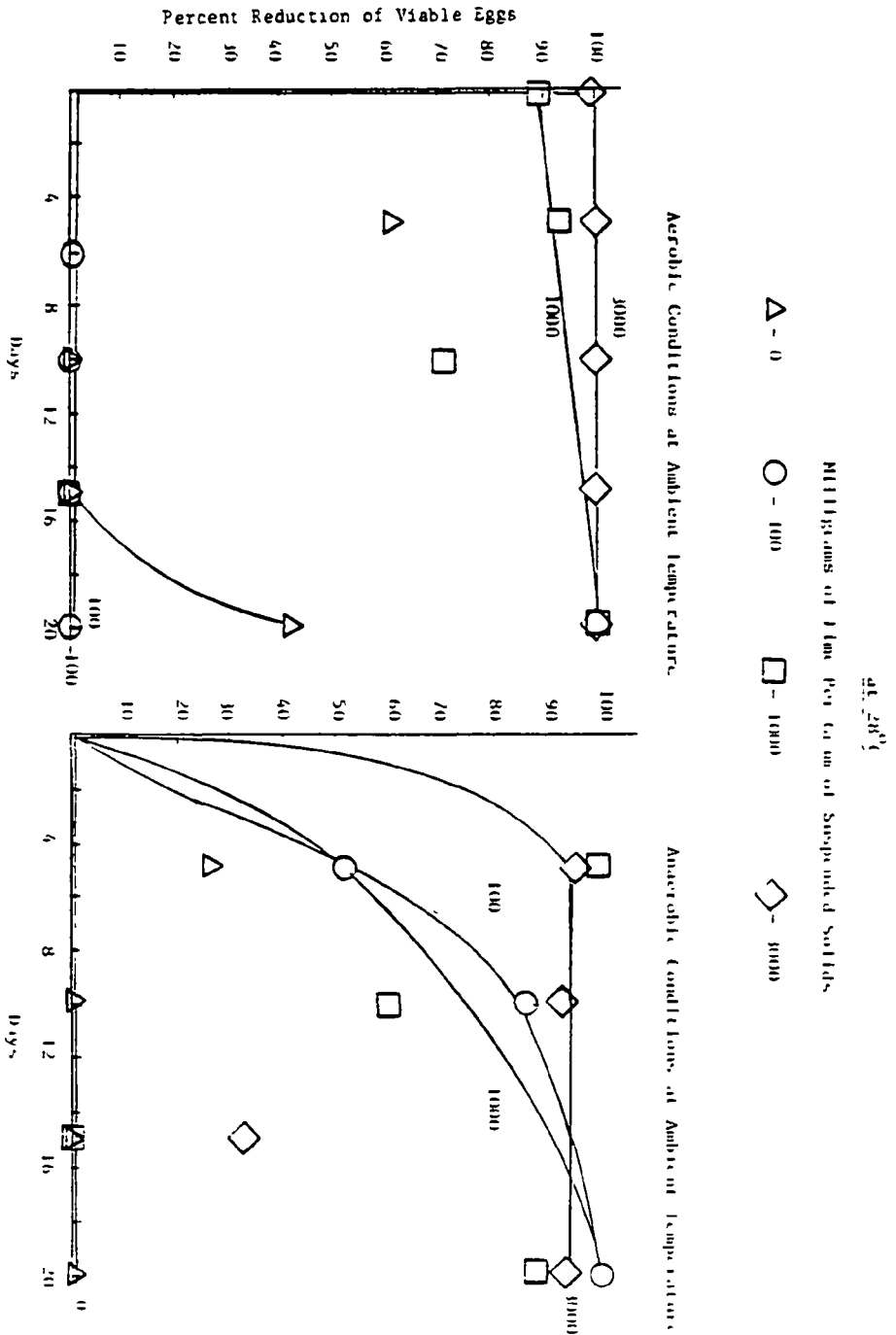


Figure 23. Effects of lime stabilization on the viability of *Aerobis* eggs (from the small intestinal contents of swine) in aerobically-digested sludge at 28°C under aerobic and anaerobic environments at ambient temperature

In the case of the 35°C aerobically-digested sludge (as shown in Figure 24), there was no apparent effect of lime on the viability of Ascaris eggs at dosages up to 3000 mg of lime per gram of dry sludge solids under anaerobic conditions in the period of 20 days. However, under aerobic conditions, a 98% reduction of viable Ascaris eggs was observed within one hour at dosages greater than 1000 mg of lime per gram of dry sludge solids, but only 77% reduction of the viable eggs were observed at a dosage of 100 mg lime per gram of dry sludge solids after 20 days. The explanation of these differences is not apparent.

Ammonia Treatment

The effects of ammonia on Ascaris and Toxocara eggs added to sludge that had been aerobically digested at 28 and 35°C and to sludge that had been anaerobically digested at 35°C was studied under anaerobic conditions at dosages of 0, 50, 500, and 5000 milligrams of ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) per gram of suspended solids. In order to keep all of the ammonium in the non-charged ammonia form, the pH was maintained greater than 12. Experiments were conducted over a period of 5 days for the anaerobically digested sludge to which sodium hydroxide had been added to maintain a pH of 12 and over a period of 15 days for aerobically digested sludge to which 3000 milligrams of lime ($\text{Ca}(\text{OH})_2$) per gram of suspended solids had been added. All flasks to which ammonium was added, were kept tightly closed to prevent the volatilization and release of ammonia.

As noted in Figure 25 and Table 25, an appreciable reduction in the recovery of viable Ascaris eggs was observed within five to 20 days for the 28°C- and 35°C-aerobically-digested sludges dosed with ammonium sulfate and lime. Even the control which had lime but no ammonia, the recovery of the viable Ascaris eggs dropped by over 95% within 5 days. It is speculated this inactivation was due to the free ammonia (endogenous free NH_3). During preliminary studies, the potential for the inactivation of parasite eggs at a free ammonia concentration at 25 mg/l was observed. In anaerobic digestion, 25 mg/l of ammonia has been found to inactivate anaerobic digestion because the sensitive microbial system is altered. Another interesting observation was the greater recovery of Ascaris eggs in the 35°C-aerobically-digested sludge than in the 28°C-aerobically-digested sludge at dosages of 50 and 500 milligrams of ammonium sulfate per gram suspended solids. An explanation of these differences is not apparent.

The results on the application of sodium hydroxide and ammonia to anaerobically digested sludge under mesophilic conditions is reported in Table 25. There was no reduction of viable Ascaris or Toxocara eggs in any of the experiments. This may be due to the fact that the free ammonia concentrations were not near the 5% concentrations reported to be necessary to inactivate Ascaris eggs in anaerobic sludges by various Russian researchers (39,76). Currently, the proper dosages of ammonia and a base for addition to

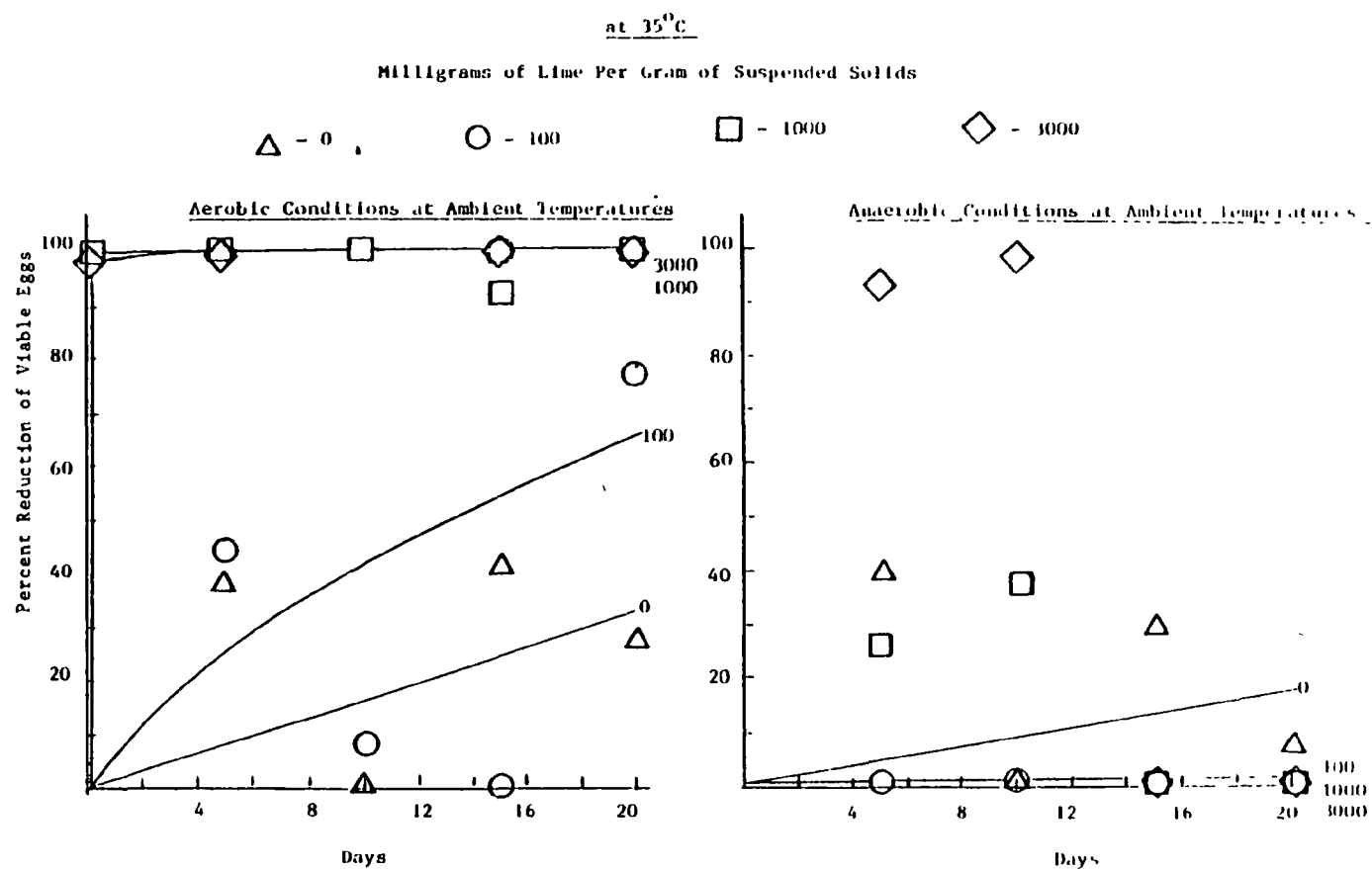


Figure 24. Effects of lime stabilization on the viability of *Ascaris* eggs (from the small intestinal contents of swine) in aerobically digested sludge at 35°C under aerobic and anaerobic environments at ambient temperature

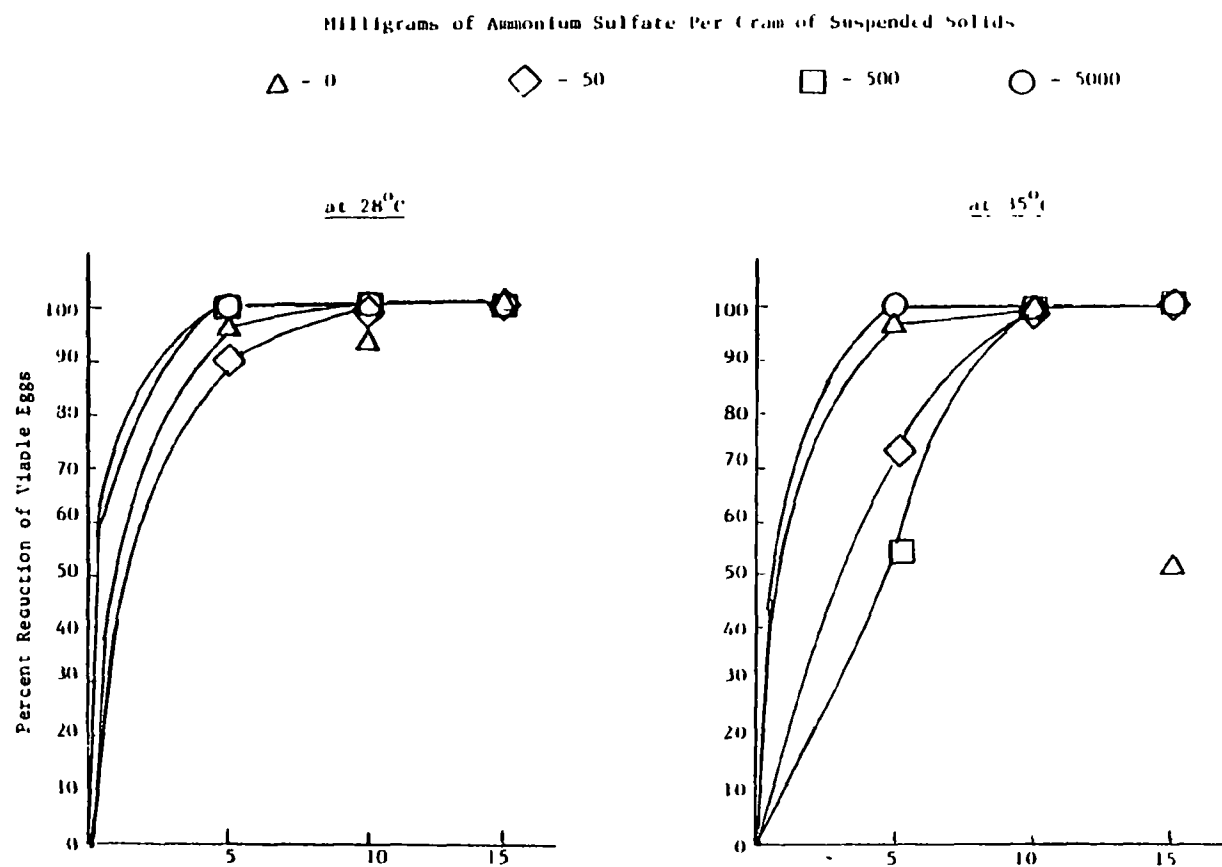


Figure 25. Effects of ammonia on the viability of *Ascaris* eggs (from the small intestinal contents of swine) in lime-stabilized aerobically-digested sludge at 28°C and 15°C under anaerobic conditions at ambient temperature (1000 mg of lime (Ca(OH)₂) per gram of suspended solids)

TABLE 25. EFFECT OF AMMONIA ON ASCARIS EGGS (FROM THE SMALL INTESTINAL CONTENTS OF SWINE) AND TOXOCARA EGGS IN 28°C OR 35°C LIME-STABILIZED AEROBICALLY-DIGESTED SLUDGE (3000 mg LIME (Ca(OH)₂) PER GRAM SUSPENDED SOLIDS UNDER ANAEROBIC CONDITIONS AND IN 35°C ANAEROBICALLY-DIGESTED SLUDGE (HELD AT pH 12 WITH SODIUM HYDROXIDE UNDER ANAEROBIC CONDITIONS) AT AMBIENT TEMPERATURES ¹

Time	Lime Stabilized Aerobically Digested Sludge ²		Caustic Stabilized Anaerobically Digested Sludge at 35°C. ³	
	at 28°C <u>Ascaris</u> Eggs	at 35°C <u>Ascaris</u> Eggs	<u>Ascaris</u> Eggs	<u>Toxocara</u> Eggs
<u>No Ammonia Dosage⁴</u>				
1 hour	-	-	-	-
5 days	96	96	0	0
10 days	93	99	-	-
15 days	100	51	-	-
<u>50 mg of Ammonium Sulfate Per Gram Susp. Solids</u>				
1 hour	-	-	-	-
5 days	90	73	0	31
10 days	99	98	-	-
15 days	100	100	-	-
<u>500 mg of Ammonium Sulfate Per Gram Susp. Solids</u>				
1 hour	-	-	-	-
5 days	98	54	0	0
10 days	100	100	-	-
15 days	100	100	-	-
<u>5000 mg of Ammonium Sulfate Per Gram Susp. Solids</u>				
1 hour	-	-	-	-
5 days	100	100	0	0
10 days	100	100	-	-
15 days	100	100	-	-

¹Percent reduction = Percent of viable eggs noted to be reduced from number of viable eggs found at one hour operation.

²pH held with 3000 mg of lime per gram suspended solids.

³pH held to 12 with sodium hydroxide.

⁴25 mg/l of free ammonia.

anaerobically-digested sludges is being restudied so that the above phenomena can be better understood.

Ultrasonication

Preliminary studies were conducted to determine the inactivation effectiveness of ultrasonication on Ascaris and Toxocara eggs. The test samples consisted of a suspension of approximately 10,000 eggs of the respective parasite in 100 ml of water, each placed in a 150 ml beaker. These samples were then exposed to various ultrasonic frequencies (kHz), intensities (wattage), and times of exposures.

The results of this preliminary study are presented in Tables 26 and 27, Table 26 shows that Ascaris eggs did not appear to be affected. Toxocara eggs, however, appeared to be significantly affected at 49 kHz and 26 watts with a 9 minute exposure and at 64 kHz and 74 watts with a 6 minute exposure. Thus, parasite egg inactivation by ultrasonication is a function of the type of parasite, ultrasonic frequency, minimum intensity and time of exposure. Table 27 indicates that an optimum intensity is required for the destruction of Toxocara eggs with the effectiveness of treatment dropping as the frequency decreases below 30 kHz and increases above 64 kHz. Again, the effect of ultrasonics on Ascaris eggs was negligible at all values of ultrasonic frequencies and intensities attempted. With an increase in intensity, the destruction of Ascaris eggs may be possible, but this hypothesis still requires study.

TABLE 26. THE EFFECT OF ULTRASONICS ON ASCARIS AND TOXOCARA EGGS
AS EVALUATED BY DIRECT OBSERVATION

Sonic Frequency (kHz)	Intensity (watts)	Exposure Time (min)	Volume Exposed (ml)	<u>Toxocara</u> Eggs	<u>Ascaris</u> Eggs
49	26	3	100	No Effect	No Effect
49	26	6	100	No Effect	No Effect
49	26	9	100	Destruction	No Effect
49	26	12	100	- ¹	No Effect
49	26	3	50	-	No Effect
36	96	3	100	No Effect	-
36	96	6	100	No Effect	-
36	96	9	100	No Effect	-
21	74	3	100	No Effect	-
21	74	6	100	No Effect	-
21	74	9	100	No Effect	-
21	74	3	50	No Effect	-
64	74	3	140	No Effect	-
64	74	3	140	No Effect	-
64	74	6	140	Destruction	-
64	74	9	140	-	-
64	17	1	100	No Effect	-
68	49	1	100	No Effect	-

¹ - = Not Done

TABLE 27. THE EFFECT OF ULTRASONICS ON THE VIABILITY OF *ASCARIS* AND *TOXOCARA* EGGS AS DETERMINED BY CULTURING TECHNIQUES.

Sample	Sonic Frequency (kHz)	Intensity (watts)	Exposure Time (min.)	Exposure Volume (ml)	<i>Toxocara</i> ¹ (% Viable)	Percent Reduction	<i>Ascaris</i> (% Viable)	Percent Reduction
Control	-	-	-	-	94.0	-	90.0	-
1	49	26	3	100	0.5	99.5	96.0	(6.7)
2	49	14	3	100	1.0	99.0	71.0	21.1
3	49	5	3	100	1.0	99.0	90.0	0.0
4	36	96	3	100	2.0	98.0	96.0	(6.7)
5	36	32	3	100	0.3	99.7	94.0	(4.4)
6	36	10	3	100	1.0	99.0	95.0	(5.6)
7	21	74	3	100	47.0	50.0	94.0	(4.4)
8	21	26	3	100	47.0	50.0	91.0	(1.1)
9	21	11	3	100	77.0	18.1	92.0	(2.2)
10	64	74	3	140	0.5	99.5	93.0	(3.3)
11	64	17	1	100	12.0	87.2	87.0	3.3
12	68	49	1	100	17.0	81.9	91.0	(1.1)

¹ Viability determined by presence or absence of larva in egg after 3-4 weeks in culture.

² () = negative percent.

REFERENCES

1. Carroll, T.E., D.L. Maase, J.M. Genco, and C.N. Ifeadi. Review of Land-spreading of Liquid Municipal Sewage Sludge. Environmental Protection Technology Series. EPA - 670/2-75-049.
2. Lund, E. Observations on the Virus Binding Capacity of Sludge. In: Proceedings of the Fifth Conference, Advances in Water Pollution Research, I-24, San Francisco, 1970, Pergamon Press, pp. 1-5, 1971.
3. Leeve, H., A. Perchet, G. Savage, S. Andrieu and R. Nguematcha. Microbiological Aspects of Sewage Treatment. In: Proceedings of the Fifth International Conference, Advances in Water Pollution Research, I-23, San Francisco, 1970, Pergamon Press, pp. 1-8, 1971.
4. Chang, S.L. Discussion of paper by Liebmann: Parasites in Sewage and the Possibilities of Their Extinction. In: Proceedings of the 2nd International Conference, Advances in Water Pollution Research. Tokyo, Japan, 1964, Pergamon Press, pp. 279-282, 1965.
5. Foster, D.H. and R.S. Englebrecht. Microbial Hazards in Disposing of Waste Water in Soil. In: Recycling Treated Municipal Wastewater and Sludge Through Forest and Crop Land. W.E. Sopper and C.T. Kardos, eds. Pennsylvania State Press, pp. 247-270, 1973.
6. Counts, C.A. and A.J. Shuckrow. Design, Development, and Evaluation of a Lime Stabilization System to Prepare Municipal Sewage Sludge for Land Disposal. U.S.E.P.A. Contract No. 68-03-0203 (draft final unpublished report), 1974.
7. Paulsrud, B. and A.S. Eikum. Lime Stabilization of Sewage Sludge. Norwegian Institute for Water Research (unpublished paper), P.O. Box 33, Oslo 3, Norway, 1970.
8. Trubnick, E.H. and P.K. Mueller. Sludge Dewatering Practice. Sewage Waste 30:1364, 1958.
9. Doyle, C.B. Effectiveness of High pH for Destruction of Pathogens in Raw Filter Cake. J. Water Pollut. Contr. Fed. 39: 1403-1409, 1967.
10. Evans, S.C. Sludge Treatment at Luton. J. Indust. Sewage Purif. 5:381, 1961.
11. Anon. How Safe is Sludge? Compost Science (March-April, 1970) p. 10, 1970.

12. Kampelmacher, E.H. and V.N. Jensen. Reduction of Bacteria in Sludge Treatment. J. Water Pollut. Contr. Fed. 44:309-313, 1972.
13. Farrell, J.B., J.E. Smith, S.W. Hathaway and R.B. Dean. Lime Stabilization of Primary Sludge. J. Water Pollut. Contr. Fed. 46:113-122, 1974.
14. Wright, W.H., E.B. Cram, and M.O. Nolan. Preliminary Observations on the Effect of Sewage Treatment Processes on the Ova and Cysts of Intestinal Parasites. Sewage Works J. 14:1274-1280, 1942.
15. Cram, E.B. The Effect of Various Treatment Processes on the Survival of Helminth Ova and Protozoan Cysts in Sewage. Sewage Works J. 15:1119-1138, 1943.
16. Cram, E.B. and D.O. Hicks. The Effect of Sludge Digestion, Drying, and Supplemental Treatment on Eggs of Ascaris lumbricoides. Proc. Helminth. Soc. Wash. 11:1-9, 1944.
17. Bond, J.O. The Risk of Ascaris Infestation from the Use of Human Sludge as Lawn Fertilizer. J. Fla. Med. Ass. 44:964-967, 1958.
18. Wang, W.-L.L. and S.G. Dunlop. Animal Parasites in Sewage and Irrigation Water. Sewage Indust. Wastes 26:1020-1032, 1954.
19. Lepak, J.W. Incidence and Transmission of Animal Parasites in Sewage and Culinary Water. Dissertation Abstr. 21(9):2835, 1961.
20. Fox, J.C. and P.R. Fitzgerald. Parasitic Organisms Present in Sewage Systems of a Large Metropolitan Sewage District. Program and Abstracts, 51st Annual Meeting, American Society of Parasitologists, pp.28-29, 1976.
21. Fox, J.C. and P.R. Fitzgerald. Parasite Content of Municipal Wastes from the Chicago Area. Program and Abstracts, 52nd Annual Meeting, American Society of Parasitologists, pp. 68-69, 1977.
22. Hays, B.D. Is there a Potential for Parasitic Disease Transmission from Land Application of Sewage Effluents and Sludges? J. Environ. Hlth. 39:424-426, 1977.
23. Theis, J.H., V. Bolton and D.R. Storm. Helminth Ova in Soil and Sludge from Twelve U.S. Urban Areas. J. Water Pollut. Contr. Fed. 50:2485-2493, 1978.
24. Jackson, G.L., J.W. Bier and R.A. Rude. Recycling of Refuse into the Food Chain: The Parasite Problem. In: Proceedings of the Conference on Risk Assessment and Health Effects of Land Application of Municipal Wastewater and Sludges. B.P. Sagik and C.A. Sorber, eds. Center for Applied Research and Technology, Univ. Texas at San Antonio, TX, 1978.
25. Akin, E.W., W. Jakubowski, J.B. Lucas and H.R. Pahren. Health Hazards Associated with Wastewater Effluents and Sludge: Microbiological Con-

siderations. Proceedings of the Conference on Risk Assessment and Health Effect of Land Application of Municipal Wastewater and Sludges. Center for Applied Research and Technology, The University of Texas at San Antonio. San Antonio, TX, 1978.

26. Morishita. Studies on Epidemiological Aspects of Ascariasis in Japan and Basic Knowledge Concerning its Control. In: Progress of Medical Parasitology in Japan, K. Morishita, Y. Komiya and H. Matsubayashi, eds. Vol. 4. Neguro Parasitology Museum, Tokyo, Japan, pp. 1-153, 1972.
27. Phillips, J. A., A.J. Harrold, G.V. Whiteman, and L. Perelmutter. Pulmonary Infiltrates, Asthma and Eosinophilia due to Ascaris suum Infestation in Man. N. Engl. J. Med. 286:1-8, 1972.
28. Beer, R.J.S. Experimental Infection of Man with Pig Whipworm. Brit. Med. J. 2:44, 1971.
29. Harper, K.L., M.D. Garfield and F.A. Ehrenford. Human Infection with Canine Whipworm. J. Indiana St. Med. Ass. 57:24-27, 1964.
30. Beaver, P.C., C.H. Synder, G.M. Carrera, J.N. Dent and J.W. Lafferty. Chronic Eosinophilia due to Visceral Larva Migrans. Pediatrics 9:7-19, 1952.
31. Wilkinson, C.P. and R.B. Welch. Intraocular Toxocara. Am. J. Ophthalmol. 71:921-930, 1971.
32. Swerczek, T.W., S.W. Nielsen, and C.F. Helmboldt. Transmammary Passage of Toxocara cati in the Cat. Am. J. Vet. Res. 32:89-92, 1971.
33. Schoenfeld, A.E., E. Githic, and N. Rosen. Granulomatous Encephalitis Due to Toxocara Larvae (Visceral Larva Migrans). Harefuah 46:337-340, 1964.
34. Perlmutter, A.D., J.B. Edlow, and S.V. Kevy. Toxocara Antibodies in Eosinophilic Cystitis. J. Pediat. 73:340-344, 1968.
35. Pappaioanou, M., C.W. Schwabe and D.M. Sard. An Evolving Pattern of Human Hydatid Disease Transmission in the United States. Am. J. Trop. Med. Hyg. 26:732-742, 1977.
36. Gamble, W.G., M. Segal, P.M. Schantz and R.L. Rausch. Alveolar Hydatid Disease in Minnesota. J. Am. Med. Ass. 241:904-907, 1979.
37. Frenkel, J.K., A. Ruiz, and M. Chinchilla. Soil Survival of Toxoplasma Oocysts in Kansas and Costa Rica. Am. J. Trop. Med. Hyg. 24:439-443, 1975.
38. Fairbairn, D. The Biochemistry of Ascaris. Exper. Parasitol. 6:491-554, 1957.

39. Chilikin, M.M. [Disinfection from Helminth Ova by Ammonium Hydroxide and Carbathion of the Mechanically Separated Solid Part of Sewage.] Abstract in: Helminthol. Abstr. Ser. A, 47(3):129, 1978.
40. Mercado-Burgos, N., R.C. Hoehn, and R.B. Holliman. Effect of Halogens and Ozone on Schistosoma Ova. J. Water Pollut. Contr. Fed. 47:2411-2419, 1975.
41. Burleson, G.R. and M. Pollard. Inactivation of Microorganisms by Ozonation or Ozonation and Sonication. (Progress report to Telecommunications Industries, Inc.) Lobund Laboratory, Notre Dame, Ind., p. 20, 1976.
42. Liebmann, H. Parasites in Sewage and the Possibilities of their Extinction. In: Proceedings of the Second International Conference. Advances in Water Pollution Research. Tokyo, 1964. Vol. 2. J.K. Baars, ed., Pergamon Press, pp. 269-279, 1964.
43. Reyes, W.L., C.W. Kruse, and M.St.C. Batson. The Effect of Aerobic and Anaerobic Digestion on Eggs of Ascaris lumbricoides var suum in Night Soil. Am. J. Trop. Med. Hyg. 12:46-55, 1963.
44. Brandon, J.R. Parasites in Soil/Sludge Systems. In: 5th National Conference on Acceptable Sludge Disposal Techniques. Cost, Benefit, Risk, Health and Public Acceptance, Orlando, Fla. Information Transfer Inc., Rockville, MD, pp. 130-133, 1978.
45. Cram, E.B. The Influence of Low Temperatures and of Disinfectants on the Eggs of Ascaris lumbricoides. J. Agr. Res. 27:167-175, 1924.
46. Nolf, L.O. Experimental Studies of Certain Factors Influencing the Development of Viability of the Ova of the Human Trichuris as Compared with those of the Human Ascaris. Am. J. Hyg. 16:288-322, 1932.
47. Alexandre, D., P. Gevaudan, J. Charrel, M.N. Mallet, A. Blanchard, and M.J. Gevaudan. La Désinfection des Boues Résiduares Urbaines. T.S.M.L. Eau, 70th yr., No. 12, pp. 547-555, Dec. 1975.
48. Sobenina, G.G. [Study of the Effect of Some Fungi on the Embryogenesis and Survival of Ascaris Ova.] Abstract In: Helminthol. Abstr. Ser. A, 47(10):440, 1978.
49. Asitinskaya, S.E. [The Role of Freshwater Gastropods in Removing Ascaris Ova from Water.] Abstract in Helminthol. Abstr., Ser. A, 48(2):89, 1979.
50. Miller, A., E. Chi-Rodriguez and R.L. Nichols. The Fate of Helminth Eggs and Protozoan Cysts in Human Feces Ingested by Dung Beetles (Coleoptera: Scarabaeidae). Am. J. Trop. Med. Hyg. 10(4):748-754, 1961.
51. Faust, E.C., P.F. Russell and R.C. Jung. Craig and Faust's Clinical Parasitology. 8th Ed. Lea & Febiger, Philadelphia, p. 890, 1970.

52. Moore, G.T., W.M. Cross, C.D. McGuire, C.S. Mollohan, N.N. Gleason, G.R. Healy, and L.H. Newton. Epidemic Giardiasis at a Ski Resort. N. Engl. J. Med. 281:402-407, 1969.
53. Shaw, P.K., R.E. Brodsky, D.O. Lyman, B.T. Wood, C.H. Hibler, G.R. Healy, K.I.E. Macleod, W. Stahl and M.G. Schultz. A Community Wide Outbreak of Giardiasis with Evidence of Transmission by a Municipal Water Supply. Ann. Intern. Med. 87:426-432, 1977.
54. Clark, C.S., E.J. Cleary, G.M. Schiff, C.C. Linnemann, Jr., J.P. Phair and T.M. Briggs. Disease Risks to Occupational Exposure to Sewage. J. Environ. Engineer. Div. 102(EE2):375-388, 1976.
55. Penfold, W.J. and H.B. Penfold. Cysticercosis bovis and its Prevention. J. Helminthol. 15:37-40, 1937.
56. Rickard, M.D. and A.J. Adolph. The Prevalence of Cysticerci of Taenia saginata in Cattle Reared on Sewage-irrigated Pasture. Med. J. Austral. 1:525-527, 1977.
57. Roberts, F.C., Jr. Experiences with Sewage Farming in Southwest United States-Arizona. Am. J. Publ. Hlth. 25:122-125, 1935.
58. Northington, C.W., S.L. Chang, and L.J. McCabe. Health Aspects of Wastewater Reuse. In: Water Quality Improvement by Physical and Chemical Processes. E.F. Gloyna and W.W. Eckenfelder, Jr. eds. University of Texas Press, Austin. TX, pp. 49-56, 1970.
59. Hammerberg, B., G.A. MacInnis, and T. Hyler. Taenia saginata Cysticerci in Grazing Steers in Virginia. J. Am. Vet. Med. Ass. 173:1462-1464, 1978.
60. Greenberg, A.E. and B.G. Dean. The Beef Tapeworm, Measley Beef, and Sewage - a Review. Sewage Indust. Wastes 30:262-269, 1958.
61. Vassilkova, Z.G., Sur la Deshelminthisation des Eaux d'Egout Epurees par Methodes Intenses. Med. Parasitol. Parasit. Dis. 5(5):657-673, 1936. Abstract In: Helminthol. Abstr. 5:139-140, 1936.
62. Silverman, P.H., and R.B. Griffiths. A Review of Methods of Sewage Disposal in Great Britain, with Special Reference to the Epizootiology of Cysticercus bovis. Ann. Trop. Med. Parasitol. 49:436-450, 1955.
63. Newton, W.L., H.J. Bennett and W.B. Figgat. Observations on the Effects of Various Sewage Treatment Processes upon the Eggs of Taenia saginata. Am. J. Hyg. 49:166-175, 1949.
64. Schmidt, C.J., I. Kugelman and E.V. Clements, III. Municipal Wastewater Reuse in the U.S. J. Water Pollut. Contr. Fed. 47(9):2229-2245, 1975.
64. Braun, R. Problems of Sludge Disposal. In: Proceedings of the Second International Conference. Advances in Water Pollution Research. Tokyo,

1964. Vol. 2. J.K. Baars, ed., Pergamon Press, pp. 217-230, 1965.
66. Golueke, C. and H.B. Gotaas. Public Health Aspects of Waste Disposal by Composting. Am. J. Publ. Hlth. 44:339-348, 1954.
 67. Wiley, B.B. and S.C. Westerberg. Survival of Human Pathogens in Composted Sewage. Appl. Microbiol. 18:994-1001, 1969.
 68. Wiley, J.S. Pathogen Survival in Composting Municipal Wastes. J. Water Pollut. Contr. Fed. 34:80-90, 1962.
 69. Brudastov, A.N., L.N. Krasnonos, V.R. Lemelev, and S.K. Kholmukhamedov. [Invasiveness of Ascaris lumbricoides Eggs for Man and Guineapigs After 10-Year Stay in the Soil.] Medskaya Parazit 39(4):447-451. Abstract in: Trop. Dis. Bull. 67(12):1498, 1970.
 70. Passman, F.J. Fate and Occurrence of Pathogens. In: Workshop on the Health and Legal Implications of Sewage Sludge Composting. Energy Resources Co., Inc., Cambridge, Mass., 1979.
 71. Melmed, L.N. and D.K. Conninos. Disinfection of Sewage Sludge with Gamma Radiation. Water S. A. 5:153-159, 1979.
 72. Grunnet, K. and S.A. Henriksen. Elimination of Ascaris suum Eggs from Sewage by Air Flotation. Nordisk Vet. Med. 29(10):458-459, 1977.
 73. Rudolfs, W., L.L. Falk and R.A. Ragotzkie. Literature Review of the Occurrence and Survival of Enteric, Pathogenic and Related Organisms in Soil, Water, Sewage, and Sludges and on Vegetation. II. Animal Parasites. Sewage Indust. Wastes 22:1417-1424, 1950.
 74. Krasnonos, L.I. [Many-Year Viability of Ascarid Eggs (Ascaris lumbricoides) in Soil of Samarkand.] Medskaya Parazit. 47: 103-106, 1978. Abstract In: Trop. Dis. Bull. 75(10):991-992, 1978.
 75. Jones, M.F. and M.S. Hummel. The Effect of Chlorine and Chloramine on Schistosoma Ova and Miracidia. Bull. (189) Nat. Inst. Hlth., pp. 173-179, 1947.
 76. Chefranova, Yu A., R.F. Davydova and L.I. Organov. [Helminthological Assessment of the Solid Part of Sewage Used as Fertilizer after Treatment with Ammonia.] Abstract in: Helminthol. Abstr. Ser. A, 47(3):129, 1978.
 77. Melmed, L.N. Disinfection of Sludge by Gamma Radiation and Microwave. City Health Department of Laboratories, Johannesburg, South Africa. Unpublished Report., 1977.
 78. Fairbairn, D. The In-vitro Hatching of Ascaris lumbricoides Eggs. Can. J. Zool. 39:153-162, 1969.

79. Rand, M.C., A.E. Greenberg and M.J. Taras. Standard Methods for the Examination of Water and Wastewater. 14 Ed., Am. Pub. Hlth. Ass., Washington, D.C., p. 1193, 1976.
80. Krige, P.R. A Survey of the Pathogenic Organisms and Helminthic Ova in Composts and Sewage Sludge. J. Inst. Sew. Purif. 1964(3):215-220, 1964.
81. Steer, A.G., J.H. Nell and S.G. Wiechers. A Modification of the Allen and Ridley Technique for the Recovery of Ascaris lumbricoides Ova from Municipal Compost. Water Res. 8:851-853, 1974.
82. Sawitz, W. The Buoyancy of Certain Nematode Eggs. J. Parasitol. 28:95-102, 1942.
83. Dada, J.O. and W.D. Lindquist. Studies on Flotation Techniques for the Recovery of Helminth Eggs from Soil and the Prevalence of Eggs of Toxocara spp. in some Kansas Public Places. J. Am. Vet. Med. Ass. 174(11):1208-1210, 1979.
84. Meyer, K.B., K.D. Miller and E.S. Kaneshiro. Recovery of Ascaris Eggs from Sludge. J. Parasitol. 64:380-383, 1978.
85. Beaver, P.C. The Detection and Identification of some Common Nematode Parasites in Man. Am. J. Clin. Path. 22:481-494, 1952.
86. DeRivas, D. An Efficient Rapid Method of Concentration for the Detection of Ova and Cysts of Intestinal Parasites. Am. J. Trop. Med. 8:63-72, 1928.
87. Monné, L. On the Histochemical Properties of the Egg Envelopes and External Cuticles of some Parasitic Nematodes. Ark. Zool., Stockholm, 9(3):93-113, 1955.
88. Sadighian, A., F. Arfaa, E. Ghadirian and K. Movafagh. Contamination with Helminth Eggs of Various Processing Stages of the Sewage Treatment Plant in Isfahan, Central Iran. Iranian J. Publ. Hlth. 5(4):180-187, 1976.
89. Gadomska, K., B. Krzysztolik, S. Wlodek, K. Ossowska-Cupryk, and T. Slomcaynski. Helminthological and Microbiological Analysis of Municipal Wastes of the City of Łódź as the Criteria for the Evaluation of the Rate of Environmental Pollution. Wiad. Parazytol. 22:503-509, 1976. Abstract in: Helminthol. Abstr. Ser. A, 46(9), No. 3877, 1977.
90. Asvin, S. Ya. and L.S. Lagutina [Assessment of the Efficacy of Mechanical Treatment of Manure as a Control Measure Against Helminths in Sewage in Smolenk.] Parazitol. Parazitar. Bolenzni 45(5):609-611, 1976.
91. Skrjabin, K.K., N.P. Shikhobalova, and I.V. Orlov. Trichocephalidae and Capillaridae of Animals and Man and the Diseases Caused by Them. Essentials of Nematodology, Vol. VI, Academy of Sciences of the U.S.S.R., Moscow, 1957. English Translation by Israel Program for Scientific Translations, Jerusalem, p. 599, 1970.

APPENDICES

APPENDIX A

PROCEDURE FOR PARASITOLOGIC EXAMINATION OF WASTE SLUDGES

Reagents

1. 1% soln (v/v) of "7X", an anionic detergent
2. ZnSO_4 soln; specific gravity 1.20, adjusted with hydrometer
3. Alcohol - Acid Soln; 35% Ethyl Alcohol + 0.27% H_2SO_4 (v/v)
4. Ether - Anhydrous
5. Siliclad (Clay Adams), for coating glassware with silicone

Materials

1. Beakers, 1000 ml., tall form, Berselius, Pyrex
2. Beakers, 1000 ml., low form, Kimax
3. Sieves, 7 in., gauges 10, 20, 50, 100, and 150
4. Funnels, powder filling, 150 mm, Nalgene
5. Applicator sticks, wooden
6. Centrifuge tubes, conical, polypropylene, 50 ml
7. Centrifuge tubes, conical, Pyrex, 15 ml
8. Pipettes, Pasteur, disposable, 5 3/4 in
9. Microscope slides, 3x2 in
10. Cover glasses, 22x30, 24x40, 24x50 mm
11. Wash bottles, plastic, 500 ml and 1000 ml
12. Aspirator carboys, 8 l, Nalgene
13. Aspirator, bottles, 4 l, Nalgene

Equipment

1. Centrifuge, IEC, with head and cups for 50 ml tubes
2. Centrifuge, Clinical model, with head for 15 ml conical tubes
3. Magnetic Stirrer
4. Ultrasonic cleaner, 11½"x5½"x6"
5. Microscope, Compound, A.O.
6. Balance, Double Beam, Harvard

Procedure

A. Thickened Sludges

1. Place measured volume of sample (50 to 100 ml depending on amount of solids) in 1000 ml low form beaker.
2. Add about 250-300 ml of 1% soln of "7X" and mix for at least 5 min. on magnetic stirrer.
3. Strain homogenized sample through the following series of sieves; 10, 20, 50, 100 and 150. One or more sieves placed on funnel over another 1000 ml beaker and sample washed through each sieve with aid of stream from a wash bottle containing 1% "7X".
4. After sample has been passed through the sieves, it is placed in a 1000 ml beaker, tall form, and allowed to settle for at least one hour.
5. Supernatant is decanted and discarded and sediment is placed in 1 (more if necessary) 50 ml centrifuge tube.
6. Sample is centrifuged at 2000 rpm for at least 5 min. and supernatant poured off.
7. Sediment is resuspended in tap water and centrifugation and decantation as in step 6 is repeated.
8. If packed volume of sediment is 7-8 ml or less, proceed as below; if volume is more than this, the sediment should be resuspended in tap water evenly divided between 2 tubes, and centrifuged as above.
9. Add ZnSO₄ soln to packed sediment, mix thoroughly with applicator sticks, increase volume to 50 ml and centrifuge at 2000 rpm for 3-5 min.
10. After centrifuge has come to a full stop and soln in tubes has

stopped swirling, pour top 30 to 40 ml of supernatant into a 400 ml beaker. During pouring off of supernatant, rotate tube in order to allow all of material floating on surface to be poured off with supernatant.

11. Dilute poured off supernatant to about 250 ml with tap water.
 12. Place diluted supernatant in 6 or more 50 ml tubes and centrifuge at 2000 rpm for 2 min.
 13. Discard supernatant by pouring off and transfer sediment to one or more 15 ml centrifuge tubes, using stream of tap water from a wash bottle to completely wash all of sediment from each tube. Fill to near top with tap water.
 14. Centrifuge at full speed in Clinical Centrifuge for 2 min., discard supernatant. If more than one tube is used combine sediment into one tube unless combined sediment would exceed 1.5 ml.
 15. Add 10 ml of alcohol-acid soln to sediment (packed sediment should not exceed 1.5 ml, if so, divide sediment into 2 or more tubes), and mix thoroughly with applicator stick.
 16. Immediately add 3 ml of ether, place rubber stopper in tube, and mix by shaking vigorously for 15 sec.
 17. Remove stopper and centrifuge for 2 min. at full speed.
 18. Use applicator stick to loosen (from the inner surface of the tube) the plug that is formed at interface of ether and alcohol-acid solutions, and pour off entire sediment and plug.
 19. Transfer sediment to one or more slides, cover with appropriate size of cover glass and examine under at least 100X magnification of compound microscope.
 20. Count each type of helminth egg and note whether eggs appear to be viable or whether they appear to be dead (non-viable). Note presence of protozoan cysts, if any, and indicate type.
 21. For reporting, convert counts to number of organisms per gram or kilogram of dry weight.
- B. Dried Sludges (i.e., from drying beds or dewatering units)
1. Weigh out a sample of 30 gm and place in a 1000 ml low form beaker and add about 300 ml of tap water.
 2. Place sample in blender and blend until thoroughly homogenized; transfer to 1000 ml beaker and add tap water until volume is about 900 ml.

3. Allow to stand overnight.
4. The next day, decant and reblend sediment as in step 2; allow to settle 1 hr or more.
5. Decant and add 1% "7X" until beaker is nearly full.
6. Allow to settle for 1 hr or more.
7. Decant, add 200-300 ml of "7X", and place on a magnetic stirrer (use 2 in. stirring bar in beaker) for 5 min.
8. Add 1% "7X" until beaker is nearly full and allow to settle for 1 hr or more.
9. Decant and pass sediment through series of sieves as in A3 (above) and follow procedure given in A4 through A21.

C. Dilute Sludges (i.e., sludges with low solid content)

1. Place measured amount of sample in 1000 ml beaker and allow to settle for at least 1 hr. Amount used initially to depend on amount of solids and in the case of very dilute samples more than 1000 ml of sample may need to be sedimented.
2. Decant supernatant and proceed as with A3 to A21 above.

GENERAL COMMENTS

After each use sieves are washed thoroughly and placed in an ultrasonic cleaner containing appropriate detergent for at least 10 minutes.

In sediments from some anaerobic sludges, the eggs of helminths are darkly stained and difficult to identify when examined microscopically. In such cases, they are bleached slightly by treating with 2% dilution of commercial bleach (Clorox) for a few minutes then washed again in water before examination.

Glassware should be coated with silicone at appropriate intervals.

TABLE A-1. NUMBER OF EGGS RECOVERED FROM SAMPLES OF ANAEROBICALLY DIGESTED THICKENED SLUDGE WITH OR WITHOUT ASCARIS EGGS ADDED¹

Parasites	Controls				500 eggs/l added (=25 eggs/50ml) 50 ml Sample		5000 eggs/l added 25 ml Sample (=125 eggs added)			50 ml Sample (=250 ml eggs added)		
	25 ml Sample		50 ml Sample		#1	#2	#1	#2	#3	#1	#2	#3
	#1	#2	#1	#2								
<u>Ascaris</u>	4	2	5	4	17 (50%) ²	16 (46%)	80 (62%)	66 (50%)	75 (58%)	185 (72%)	153 (59%)	178 (69%)
<u>Toxocara</u>	3	1	2	3	4	1	2	2	2	2	4	4
<u>T. trichiura</u>	0	1	4	0	4	2	1	0	1	2	0	5
<u>T. vulpis</u>	0	1	2	1	2	1	1	1	2	1	1	3
<u>H. diminuta</u>	0	1	0	0	2	2	0	0	0	0	0	0

1 Samples examined 3 wks after Ascaris eggs were added to experimental flasks containing thickened anaerobic sludge control and experimental flasks maintained at R1.

2 Approximate percent recovery of Ascaris eggs added.

TABLE A-2. NUMBER OF EGGS RECOVERED FROM SAMPLES (100 ml EACH) OF ANAEROBICALLY DIGESTED SLUDGE WITH OR WITHOUT (CONTROLS) ASCARIS EGGS ADDED¹

		Controls				200 <u>Ascaris</u> eggs/100 ml added											At 1nd Ave. Ave Z Recovery	
		At Start 2	At End 3			At Start 2	At 1nd 3											
			1	2	3		1	2	3	4	5	6	7	8	9			
<u>Ascaris</u>	V	20	16	26	12	84	33	34	29	32	39	28	52	26	52	45.1	12%	
	NV		5	4	2		5	12	8	8	4	16	10	9	9			
<u>Toxocara</u>	V	8	1	1	2	12	5	0	3	0	1	3	1	4	2			
	NV		5	7	7		8	9	9	6	9	9	13	15	15			
<u>I. trichiura</u>	V	8	1	2	5	13	7	3	5	0	5	2	3	2	1			
	NV		1	0	0		3	1	4	2	4	0	1	0	0			
<u>T. vulpis</u>	V	3	2	3	1	4	2	3	1	4	5	1	0	3	2			
	NV		0	1	1		0	1	0	1	0	0	0	0	0			
<u>T. diminuta</u>	V	1	0	0	0	2	0	0	0	0	0	0	0	0	0			
	NV		2	0	0		1	0	0	0	1	1	3	0	0			

- 1 Ascaris eggs added on 17 Feb. 1978 to Anaerobic sludge
- 2 Samples taken one day after eggs were added to experimental flasks.
- 3 Samples taken 25 days after eggs were added to experimental flasks.

TABLE A-3. NUMBER OF EGGS RECOVERED FROM SAMPLES (100 ml EACH) OF AEROBICALLY DIGESTED SLUDGE WITH OR WITHOUT (CONTROLS) ASCARIS EGGS ADDED¹

		Controls				200 <u>Ascaris</u> eggs/100 ml added														At End Ave.	Ave. % Recovery
		At Start 2	At End 3			At Start 2	At End 3														
			1	2	3		1	2	3	4	5	6	7	8	9	10					
<u>Ascaris</u>	V	7	14	12	6	42	24	28	28	29	42	38	18	43	38	42	36.7	13%			
	NV	0	1	1	1	0	4	5	2	4	1	7	1	1	5	2					
<u>Toxocara</u>	V	0	1	0	1	0	1	0	0	1	2	1	0	1	1	2					
	NV	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0					
<u>T. trichiura</u>	V	0	3	1	2	0	3	2	3	2	2	1	2	1	1	3					
	NV	0	1	0	1	0	1	1	1	0	0	1	1	0	1	1					
<u>E. vulpis</u>	V	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1					
	NV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					

1 Ascaris eggs added on 17 Feb. 1978 to Aerobic Sludge

2 Samples taken one day after eggs were added to experimental flasks

3 Samples taken 25 days after eggs were added to experimental flasks

APPENDIX B
RESULTS OF FIELD INVESTIGATION

TABLE B-1. FALL (NOVEMBER & DECEMBER, 1977) EXAMINATION FOR PARASITES IN SLUDGES FROM MUNICIPAL TREATMENT PLANTS OF THE SOUTHERN UNITED STATES

Plant-Site	Actual Size Sample Examined grams dry weight	<u>Ascaris</u>		<u>T. trichiura</u>		<u>T. vulpis</u>		<u>Toxocara</u>		<u>H. diminuta</u>	
		v	nv	v	nv	v	nv	v	nv	v	nv
		eggs/Kg ²		eggs/Kg		eggs/Kg		eggs/Kg		eggs/Kg	
1. 1 ^o Sludge	5.0	10,000	0	1,800	0	0	0	800	0	1,200	200
Anaerobic Digester	5.0	12,000	3,400	400	0	1,400	600	0	1,000	0	600
Drying Bed	9.1	1,500	1,800	0	770	110	330	0	440	0	330
2. Anaerobic Digester	6.4	500	300	0	0	160	0	310	0	0	470
Drying Bed	7.2	1,800	1,700	0	0	280	410	700	2,400	0	1,300
3. Aerobic Digester ¹	1.4	7,700	0	0	0	700	0	3,500	0	0	0
4. Anaerobic Digester	2.5	1,200	8,200	0	400	3,300	0	0	2,400	0	820
Drying Bed	SAMPLE LOST										
+ 5. 1 ^o Sludge	SAMPLE LOST										
Drying Bed	(Drying bed sample was supernatant from digester, thus not applicable)										
6. Activated Sludge ¹	0.9	4,300	0	0	0	0	0	0	0	0	0
Drying Bed	16.6	0	200	0	0	0	0	0	0	0	0
7. Activated Sludge ¹	7.2	18,000	700	560	0	0	140	840	0	0	140
Drying Bed	5.4	220,000	10,000	930	3,300	930	0	560	1,300	0	370

(continued)

TABLE B-1. (continued)

Plant-Site	Actual Size Sample Examined grams dry weight	<u>Ascaris</u>		<u>T. trichiura</u>		<u>I. vulpis</u>		<u>Toxocara</u>		<u>H. diminuta</u>	
		v	nv	v	nv	v	nv	v	nv	v	nv
		eggs/Kg ²		eggs/Kg		eggs/Kg		eggs/Kg		eggs/Kg	
8. 1 ^o Sludge	5.0	7,800	0	0	0	400	0	1,800	200	2,800	5,000
Drying Bed	22.3	0	0	0	0	50	0	50	90	0	0
+ 9. 1 ^o Sludge	16.6	1,900	60	0	0	0	60	120	0	240	240
Anaerobic Digester	5.8	7,200	500	0	0	0	0	690	0	170	520
10. 1 ^o Sludge	3.2	950	0	0	0	310	0	320	0	320	0
Drying Bed	8.9	2,300	1,200	0	110	1,100	0	450	450	0	1,900
11. 1 ^o Sludge	1.1	2,700	0	0	0	0	0	2,700	0	910	0
Drying Bed	23.4	40	260	0	0	130	0	0	260	0	40
+ 12. 1 ^o Sludge	LOST IN WRECK										
Drying Bed											
13. Activated Sludge ¹	0.7	0	0	0	0	0	0	1,400	0	0	0
Aerobic Digester ¹	1.2	800	2,500	0	0	1,700	0	830	3,300	0	0
14. 1 ^o Sludge											
Drying Bed											
+ 15. 1 ^o Sludge	2.2	56,000	1,400	2,300	0	460	0	3,700	0	2,300	910
2 ^o Anaerobic Digester	1.1	3,700	0	0	0	0	0	930	0	930	0
16. Raw Influent ¹	0.9	12,000	0	0	0	0	0	0	0	5,500	2,200
Drying Bed	14.3	4,400	11,000	0	350	490	0	0	490	70	5,500
17. Aerobic Digester ¹	0.7	29,000	4,400	1,500	0	0	0	1,500	0	1,500	0
Lagoon	4.8	2,300	15,000	0	210	1,500	420	0	2,300	0	420
18. Activated Sludge ¹	6.8	10,000	0	7,700	590	1,500	0	440	0	150	300
Drying Bed	8.0	35,000	16,000	15,000	21,000	4,000	880	250	1,700	0	250
19. Raw Influent ¹	1.1	8,000	1,800	6,200	0	890	0	2,700	0	1,800	3,600
Anaerobic Digester	10.1	20,000	6,000	7,000	2,900	1,900	200	1,100	400	400	3,000

(continued)

TABLE B-1. (continued)

Plant-Site	Actual Size Sample Examined grams dry weight	<u>Ascaris</u>		<u>T. trichiura</u>		<u>T. vulpis</u>		<u>Lo. osili</u>		<u>H. diminuta</u>	
		v	nv	v	nv	v	nv	v	nv	v	nv
		eggs/Kg ²		eggs/Kg		eggs/Kg		eggs/Kg		eggs/Kg	
+ 20. Activated Sludge	1.1	2,800	0	0	0	0	0	900	960	950	0
21. 1 ^o Sludge	1.6	640	0	640	0	0	0	0	0	0	0
Drying Bed	28.5	0	70	0	800	0	0	0	0	0	0
22. Activated Sludge ¹	2.9	1,400	0	0	0	0	0	1,000	0	1,000	0
Aerobic Digester	1.8	6,300	0	0	570	570	0	1,700	0	570	570
Centrifuged Aerobic	5.2	6,900	580	380	0	570	0	2,100	770	0	390
+ 23. 1 ^o Sludge	1.9	2,600	530	0	0	0	0	530	0	0	0
Drying Bed	20.1	350	0	0	0	50	0	250	50	250	0
24. 1 ^o & Activated Sludge	3.0	14,000	990	0	0	0	0	990	0	0	0
Drying Bed	13.2	830	300	80	80	0	0	0	80	0	0
25. 1 ^o Sludge	2.5	0	0	0	0	400	0	1,200	0	0	0
Thickened Aerobic	1.4	700	0	0	0	0	0	700	0	0	0
Drying Bed	25.3	0	0	0	0	0	160	0	0	0	0
26. Raw Influent	1.6	5,800	0	0	0	1,300	0	650	0	0	1,500
Imhoff Digester	1.8	9,600	570	0	570	1,100	570	2,800	570	0	0
Drying Bed	13.8	650	150	0	0	70	0	0	70	0	290
+ 27. 1 ^o Sludge	3.7	0	0	270	0	0	0	820	270	270	0
Drying Bed	13.8	650	150	0	0	0	40	0	0	0	0
28. Contact Stabilized	1.7	600	0	0	0	0	0	1,800	1,200	0	0
Drying Bed	27.1	0	40	0	40	0	110	0	100	0	0
29. Thickened Activated	3.2	1,600	0	0	0	950	0	950	0	210	0
Drying Bed	28.1	150	70	0	40	180	40	250	70	0	0
30. 1 ^o Sludge	1.7	0	0	0	0	0	0	1,200	0	0	610
Drying Bed	6.6	0	1,200	0	0	300	0	0	150	0	460

(continued)

TABLE B-1. (continued)

Plant-Site	Actual Size Sample Examined grams dry weight	<u>Ascaris</u>		<u>T. trichiura</u>		<u>T. vulpis</u>		<u>Ioxocara</u>		<u>H. diminuta</u>	
		v	nv	v	nv	v	nv	v	nv	v	nv
		eggs/Kg ²		eggs/Kg		eggs K/g		eggs K/g		eggs K/g	
31. 1 ^o Sludge	2.9	340	340	0	0	0	0	1,000	340	0	340
Activated Sludge	2.3	0	0	0	0	0	0	1,300	0	0	860
Drying Bed	22.5	0	0	0	0	0	0	0	40	0	0
+ 32. Raw Influent ¹	0.3	9,400	3,100	3,100	0	0	0	0	0	0	0
1 ^o Sludge	3.1	1,300	330	0	0	0	0	0	0	0	0
Drying Bed	20.7	530	30	0	0	100	0	480	50	0	50
+ 33. 1 ^o Sludge	24.7	0	0	0	0	0	0	200	40	0	40
Drying Bed	22.0	0	0	0	0	0	0	0	0	0	0
34. 1 ^o Sludge	4.5	660	0	440	0	440	0	1,300	0	880	440
Drying Bed	20.0	0	50	0	0	400	100	0	0	0	0
+ 35. Drying Bed	20.8	0	0	0	0	0	340	0	50	0	0
+ 36. 1 ^o Sludge	3.1	9,800	0	0	0	330	0	330	0	980	0
Drying Bed	20.0	50	2,300	0	50	0	50	50	100	0	100
37. 1 ^o Sludge	11.7	9,300	430	6,600	1,000	90	0	1,900	0	0	90
Drying Bed	22.4	670	8,300	490	5,000	50	0	0	0	0	50
38. 1 ^o Sludge	4.1	240	0	0	0	0	0	490	0	0	0
Drying Bed	27.9	0	0	0	0	40	0	0	0	0	0

v= viable nv= nonviable

¹ five gallons of sewage settled overnight, then decanted; sample taken from remaining sediment² eggs/kilogram dry weight of sample

+ plan to drop from study

TABLE B-2. MISCELLANEOUS PARASITES (NOT INCLUDED IN TABLE 1) FOUND IN SLUDGES FROM MUNICIPAL TREATMENT PLANTS (FALL - NOVEMBER AND DECEMBER, 1977)

Plant-Site	Parasites
1. Anaerobic Digester	<u>Trichosomoides</u> - like eggs, N.V. 200/kg d.w
Drying bed	<u>Capillaria</u> sp. eggs (shell with striations) N.V. 110/l p.d w.
7. Drying bed	<u>Eimeria</u> oocysts - numerous
10. 1 st Sludge	<u>Ascaridia</u> - like eggs, V. 2,500/kg.d.w. <u>Eimeria</u> oocysts - numerous
Drying bed	<u>Capillaria</u> sp. eggs (shell with striations), N.V. 110/kg d.w. <u>Ascaridia</u> - like eggs, N V 5700/kg.d w <u>Eimeria</u> oocysts, N.V. - numerous
11. 1 st Sludge	<u>Ascaridia</u> - like eggs V. 51,000/kg.d w <u>Ascaridia</u> - like eggs, N V 2,700/kg d.w. <u>Eimeria</u> oocysts, V - numerous
Drying bed	<u>Ascaridia</u> - like eggs, N.V. 6,300/kg d w. <u>Capillaria</u> sp. eggs (shell with striations), N.V. 40/kg d.w. <u>Coccidia</u> oocysts, 2 or more types,numerous
+ 15. 1 st Sludge	<u>Hymenolepis nana</u> eggs, N.V. 900/kg.d.w. <u>Ascaridia</u> - like eggs, V. 1,400/kg d.w
16. Drying Bed	<u>Capillaria hepatica</u> - like eggs (shell with pits), V. 210/kg d w. <u>Capillaria</u> sp. eggs (shell with striations), V. 70/kg.d.w <u>Capillaria</u> sp. eggs (shell with striations), N.V. 490/kg.d.w <u>Trichosomoides</u> - like eggs, N V. 70/kg.d.w
17. Lagoon	<u>Ascaridia</u> - like eggs, N V 1,900/kg.d w.

(continued)

TABLE B-2 (continued)

18.	Activated Sludge ¹	<u>Capillaria</u> sp. eggs (shell with shallow pits), V. 150/kg d.w
	Drying bed	<u>Trichosomoides</u> - like eggs, N.V. 120/kg d.w <u>Capillaria</u> sp. eggs (shell with striations), N.V. 120/kg d.w
19.	Raw Influent ¹	<u>Capillaria</u> sp. eggs (shell with striations), V. 400/kg d.w.
	Anaerobic digester	<u>Ascaridia</u> - like eggs, N.V. 110/kg d.w <u>Capillaria</u> sp. eggs (shell with striations), V. 300/kg d.w <u>Capillaria</u> sp. eggs (shell with striations), N.V. 100/kg d.w <u>Cruzia</u> - like eggs, V. 100/kg d.w.
	Activated Sludge ¹	<u>Ascaridia</u> - like eggs, V. 350/kg.d.w. <u>Ascaridia</u> - like eggs, N.V. 100/kg d.w
	Centrifuged Aerobic	<u>Capillaria</u> sp. eggs (shell with striations), V. 570/kg d.w
+ 23.	Drying bed	coccidia oocysts - V. few
24.	10 & Activated Sludge	Acanthocephalan eggs (<u>Macroranthorhynchus?</u>), V. 130/kg.d.w
25.	10 Sludge	<u>Toxascaris</u> - like eggs, V. 410/kg.d.w.
	Thickened Aerobic	<u>Capillaria</u> sp. eggs (shell with pits), V. 700/kg.d.w.
	Drying bed	<u>Capillaria</u> sp. eggs (shell with striations), V. 40/kg.d.w
26.	Imhoff Digester	<u>Entamoeba coli</u> - like cysts, N.V. 6,200/kg d.w
+ 27.	10 Sludge	<u>Taenia</u> sp. eggs, N.V. 270/kg.d.w.
	Drying bed	<u>Capillaria</u> sp. eggs (shell with striations), N.V. 40/kg.d.w

(continued)

TABLE B-2. (continued)

28.	1 ⁰ Sludge	<u>Entamoeba coli</u> - like cysts, N.V.	few
30.	1 ⁰ Sludge	<u>Entamoeba coli</u> - like cysts, N.V. <u>Giardia</u> sp. cysts, N.V.	few few
31.	Activated Sludge	<u>Entamoeba</u> sp. cysts, N.V.	few
+ 32.	Raw Influent ¹	<u>Entamoeba coli</u> - like cysts, N.V.	few
+ 36.	1 ⁰ Sludge	<u>Entamoeba coli</u> - like cysts, N.V.	few
	Drying bed	<u>Trichosomoides</u> - like eggs, N.V.	50/kg d w
37.	1 ⁰ Sludge	<u>Giardia</u> sp. cysts, N.V. <u>Entamoeba coli</u> - like cysts, N.V.	few few
	Drying bed	<u>Capillaria</u> sp. eggs (shell with striations), N.V.	40/kg d w.

V. = Viable

N.V. = Nonviable

¹ five gallons of sewage settled overnight, then decanted; sample taken from remaining sediment
+ plan to drop from survey.

TABLE B-J. SLUDGE CHARACTERISTICS OF SAMPLES COLLECTED IN
FALL (NOVEMBER AND DECEMBER, 1977)

Plant Site	Total Solids mg/l (**mg/g)	Total Volatile Solids mg/l (**mg/g)	Percent Moisture Content	Chemical Oxygen Demand mg/g	Kjeldahl Nitrogen mg/g	pH	Oxidation Reduction Potential
1. 1 st Sludge Anaerobic Digester Drying Bed	50,187 100,192 **302	29,150 55,946 **66	70	896 235	18 16 5	5.4 7.2 6.9	-7 -137 -43
2. Anaerobic digester Drying Bed	64,220 **240	29,458 **122	76	1,072 295	33 23	7.0 7.1	-102 -35
3. Aerobic Digester ¹	14,310	11,300		1,088	74	6.5	-76
4. Anaerobic Digester Drying Bed	47,094 **773	29,538 **503	23	991	31	6.7	-62
+ 5. 1 st Sludge Drying Bed	31,792 Drying Bed sample was supernatant from digester, thus not applicable	24,736			29	5.7	-14
6. Activated Sludge ¹ Drying Bed	18,636 **553	12,984 **226	45	516 384	21 19	6.7	-61
7. Activated Sludge ¹ Drying Bed	7,172 **180	5,120 **171	83	334	41 53	6.9	
8. 1 st Sludge Drying Bed	49,984 **744	45,200 **483	76	1,077 505	31 20	5.7	
+ 9. 1 st Sludge Anaerobic Digester	16,398 58,178	15,730 20,316			6	6.7 6.8	-28 -86
10. 1 st Sludge Drying Bed	43,600 58,178	42,186 20,186	70	1,371 802	32 38	5.7 5.0	
11. 1 st Sludge Drying Bed	22,000 **781	13,570 **574	22	1,122 278	13 11	5.8	
+ 12. 1 st Sludge Drying Bed	LOST IN WRECK **833	**644	17				
13. Activated Sludge ¹ Aerobic Digester ¹	17,356 12,000	4,796 2,600		275 710	19 47	7.12 6.6	

(continued)

TABLE B-3. (continued)

Plant Site	Total Solids	Total Volatile Solids	Percent Moisture Content	Chemical Oxygen Demand	Kjeldahl Nitrogen	pH	Oxidation Reduction Potential
	mg/l (**mg/g)	mg/l (**mg/g)		mg/l	mg/g		
14. 1 ⁰ Sludge Drying Bed	40,612 LOST IN WRECK	31,728		1,298	36	5.7	-15
+ 15. 1 ⁰ Sludge 2 ⁰ Anaerobic Digester	29,234 21,400	22,294 17,158				5.8 6.0	-17
16. Raw Influent ¹ Drying Bed	18,200 **475	10,626 **177	52	980 488	19 21	5.7	
17. Aerobic Digester ¹ lagoon	13,800 **160	9,644 **41	84	921 365	33 15	5.7 7.0	
18. Activated Sludge ¹ Drying Bed	67,988 **267	44,272 **140	73	572 579	29 27	5.5	
19. Raw Influent ¹ Anaerobic Digester	25,000 100,872	13,056 39,748		106 572		7.0 6.2	
+ 20. Activated Sludge	3,500	2,784			21	6.8	
21. 1 ⁰ Sludge Drying Bed	856 **950	340 **413	5	1,127 335	65	7.2	
22. Activated Sludge ¹ Aerobic Digester Centrifuged Aerobic	28,760 17,484 **74	19,436 10,604 **91	83	1,121 1,104 438	68 45 39	6.2 7.2 6.7	
+ 23. 1 ⁰ Sludge Drying Bed	4,768 **669	3,252 **390	33	602	15 17	7.0	
24. 1 ⁰ & Activated Sludge Drying Bed	30,360 **440	22,804 **155	56	1,350 775	32 21	6.2	
25. 1 ⁰ Sludge Thickened Aerobic Drying Bed	24,464 14,220 **843	16,968 8,552 **298	16	1,853 106 581	55 51 3	5.8 6.7	
26. Raw Influent ¹ Imhoff Digester Drying Bed	15,600 17,700 **459	10,956 10,624 **214	54	7 617 764	49 51 26	6.7	

(continued)

TABLE B-3 (continued)

Plant Site	Total Solids	Total Volatile Solids	Percent Moisture Content	Chemical Oxygen Demand	Kjeldahl Nitrogen	pH	Oxidation Reduction Potential
	mg/l (**mg/g)	mg/l (**mg/g)		mg/g	mg/g		
+ 27. 1 ⁰ Sludge Drying Bed	36,724 **848	29,928 **313	15	1,476	37 9	5.6	
28. Contact Stabilized Drying Bed	16,460 **902	11,824 **458	10	1,173 811	65 50	6.4	
29. Thickened Activated Drying Bed	31,756 **937	25,504 **239	6	1,392 349	62 15	6.4 7.5	
30. 1 ⁰ Sludge Drying Bed	3,880 **220	2,564 **72	78	689	36	6.6	
31. 1 ⁰ Sludge Drying Bed	29,364 **750	20,420 **280	25	1,199 367	32 12	6.0	
+ 32. Raw Influent	828	460				7.7	
1 ⁰ Sludge Drying Bed	30,500 **690	17,192 **163	31	413	7 14	7.0	
+ 33. 1 ⁰ Sludge Drying Bed	247,228 **732	229,852 **244	27	167	12	5.8	
34. 1 ⁰ Sludge Drying Bed	45,444 **666	30,536 **260	33	877 177	27 20	5.8	
+ 35. Drying Bed	**693	**168	31		12		
+ 36. 1 ⁰ Sludge Drying Bed	30,500 **665	17,192 **145	34	1,006	40 10	6.3	
37. 1 ⁰ Sludge Drying Bed	116,476 **745	36,796 **64	25	378 142	7 6	5.9	
38. 1 ⁰ Sludge Drying Bed	41,132 **930	33,372 **439	7	884 823	43 25		

1 Five gallons of sewage settled overnight, then decanted; sample taken from remaining sediment.
+ plan to drop from study.

TABLE B-4. EXAMINATION FOR PARASITES IN SLUDGES FROM MUNICIPAL TREATMENT PLANTS
OF THE SOUTHERN UNITED STATES

Plant Site	Actual Size Sample Examined grams dry weight	<i>Ascaris</i>		<i>T. trichiura</i>		<i>T. vulpis</i>		<i>Toxocara</i>		<i>H. diminuta</i>	
		v	nv	v	nv	v	nv	v	nv	v	nv
		eggs/Kg 2	eggs/Kg 2	eggs/Kg 2	eggs/Kg 2	eggs/Kg 2	eggs/Kg 2	eggs/Kg 2	eggs/Kg 2	eggs/Kg 2	eggs/Kg 2
1 1 ^o Sludge Drying Bed	6.3	7,100	0	160	160	630	0	3,300	160	310	470
	13.7	4,500	5,300	0	1,000	1,400	600	0	1,400	0	430
2 1 ^o Sludge Drying Bed	3.5	280	560	0	0	280	0	1,700	280	560	280
	23.8	0	130	0	0	40	40	0	40	0	0
3 Aerobic Digester ¹	2.3	4,800	0	0	0	440	0	6,600	0	0	0
4 1 ^o Sludge Drying Bed	4.2	480	4,600	0	240	2,200	720	1,700	0	240	1,200
	7.9	1,900	16,000	510	4,500	8,200	2,300	0	630	0	2,300
6. Activated Sludge ¹ Drying Bed	1.3	3,900	0	0	0	0	0	4,700	0	780	0
	16.6	540	120	0	0	0	60	0	0	0	0
7 Activated Sludge ¹ Drying Bed	1.0	130,000	12,000	980	0	0	0	0	0	0	0
	7.1	120,000	17,000	2,500	1,700	560	280	420	1,400	0	140
8 1 ^o Sludge Drying Bed	5.5	22,000	0	0	0	550	0	4,000	180	1,800	3,300
	16.2	60	190	0	60	0	0	0	0	0	120
10 1 ^o Sludge Drying Bed	3.0	3,400	670	0	0	670	0	2,000	0	340	340
	15.4	3,700	580	0	130	780	450	450	580	60	1,200
11. 1 ^o Sludge ¹ Drying Bed	8.2	1,800	0	0	0	370	0	850	610	0	240
	14.8	200	610	0	200	470	70	0	400	0	0
13. 1 ^o & Activated Sludge ¹ Aerobic Digester ¹ Drying Bed	1.9	0	0	0	0	1,100	0	1,600	0	0	0
	1.2	0	0	0	0	1,700	0	3,400	0	0	0
	3.0	0	0	0	0	0	0	0	0	0	0
14. 1 ^o Sludge Drying Bed	2.1	0	0	0	0	0	0	2,400	0	0	0
	12.5	4,900	6,500	0	0	160	0	80	400	0	2,300
16 1 ^o Sludge Drying Bed	3.0	8,300	1,700	0	0	330	0	660	0	660	5,000
	23.0	610	3,300	40	300	780	430	40	430	0	40
17 Contact Stabilized ¹ Aerobic Digester ¹	2.9	7,300	700	0	0	0	0	1,700	0	0	0
	2.2	5,600	460	0	0	930	0	1,900	0	0	0

(continued)

TABLE B-4. (continued)

Plant Site	Actual Size Sample Examined grams dry weight	Ascaris		T. trichiura		T. vulpina		Toxocara		H. diminut.	
		v	nv eggs/Kg ²	v	nv eggs/Kg ²	v	nv eggs/Kg ²	v	nv eggs/Kg ²	v	nv eggs/Kg ²
18 1 ^o Sludge	6.4	19,000	790	10,000	470	790	0	2,000	0	160	0
Drying Bed	18.2	1,400	50	380	490	710	110	50	0	0	50
19 1 ^o Sludge	9.1	12,000	770	3,100	110	1,700	0	310	0	270	770
Anaerobic Digester	11.9	13,000	1,300	3,400	1,000	1,100	80	1,100	80	0	1,300
21 1 ^o Sludge	3.1	1,600	0	650	0	3,600	0	2,000	0	650	650
Drying Bed	21.5	0	190	0	170	0	50	0	0	0	0
22 Activated Sludge 1	1.3	2,400	0	790	790	0	0	4,000	0	0	790
Aerobic Digester	3.1	3,200	0	0	0	690	0	1,300	0	170	0
Vacuum Activated	3.8	260	0	0	0	0	260	0	0	0	260
Centrifuged Aerobic	4.6	430	0	0	0	0	0	270	0	270	0
24 1 ^o & Activated Sludge	3.7	11,000	820	0	270	0	0	270	0	0	570
Drying Bed	15.2	660	130	130	70	0	0	0	0	0	0
25 1 ^o Sludge	8.4	0	0	0	120	480	0	950	0	0	0
Thickened Activated	1.9	0	520	0	0	0	0	520	0	0	0
Drying Bed	22.5	0	0	0	0	0	0	0	0	0	0
26 Imhoff Digester	6.8	2,800	0	0	300	300	0	600	0	0	540
Drying Bed	14.0	140	70	0	0	70	0	70	140	0	0
28 Contact Stabilized	1.6	1,200	0	0	0	0	0	1,900	0	0	0
Drying Bed	16.8	240	180	0	0	60	180	60	300	0	0
29 Thickened Activated	4.7	630	0	0	0	210	0	1,100	420	210	0
Drying Bed	13.6	880	510	0	0	220	70	70	220	0	0
30 1 ^o Sludge	4.2	240	0	0	0	0	0	480	0	480	0
Drying Bed	17.6	0	0	0	0	0	0	60	0	0	0
31 1 ^o Sludge	1.9	0	0	0	0	0	0	1,000	0	0	0
Drying Bed	15.2	200	70	0	70	70	0	70	0	0	0
36 1 ^o Sludge	3.3	4,500	1,200	600	0	300	0	1,500	0	0	2,500
Drying Bed	18.7	50	0	0	0	380	110	50	0	0	0

(continued)

TABLE B-4. (continued)

Plant Site	Actual Size Sample Examined grams dry weight	<u>Ascaris</u>		<u>T. trichiura</u>		<u>E. vulpis</u>		<u>Toxocara</u>		<u>H. diminuta</u>	
		v	nv	v	nv	v	nv	v	nv	v	nv
		eggs/Kg ²		eggs/Kg ²		eggs/Kg ²		eggs/Kg ²		eggs/Kg ²	
37. 1 ⁰ Sludge	4.9	16,000	1,000	9,100	410	210	0	210	0	0	0
Drying Bed	16.1	38,000	4,800	47,000	16,000	120	190	370	190	0	120
38. 1 ⁰ Sludge	3.5	0	0	0	0	280	0	850	280	0	0
Drying Bed	26.7	40	0	70	0	0	40	0	40	0	0

n = viable

nv = nonviable

1 Five gallons of sewage settled overnight, then decanted; sample taken from remaining sediment

2 eggs/kilogram dry weight of sample

TABLE B-5. MISCELLANEOUS PARASITES (NOT INCLUDED IN TABLE 1) FOUND IN SLUDGES FROM MUNICIPAL TREATMENT PLANTS (WINTER - FEBRUARY AND MARCH, 1978)

<u>Plant-Site</u>	<u>Parasites</u>
1. 1 ⁰ Sludge	<u>Giardia</u> sp. cysts, N.V. few <u>Trichosomoides</u> -like eggs, V 160/kg d.w.
Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations), N.V. 70/kg.d.w.
2. 1 ⁰ Sludge	<u>Capillaria</u> sp. eggs (shell with fine pits), V. 280/kg d.w. <u>Capillaria</u> sp. eggs (shell with striations), V. 280/kg.d w
Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations), N.V. 40/kg.d w
3. Aerobic Digester ¹	<u>Entamoeba coli</u> -like cysts, N.V. 2,200/kg d.w.
4. 1 ⁰ Sludge	<u>Entamoeba coli</u> -like cysts, N.V. 960/kg.d.w.
7. Activated Sludge ¹	<u>Entamoeba coli</u> -like cysts, N.V. 2,000/kg d.w.
Drying Bed	<u>Coccidia</u> oocysts, N.V. few
8. 1 ⁰ Sludge	<u>Capillaria</u> sp. eggs (shell with striations), V. 730/kg.d w. <u>Trichosomoides</u> -like eggs, V. 180/kg d.w.
Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations), V. 120/kg.d.w.
10. 1 ⁰ Sludge	<u>Entamoeba coli</u> -like cysts, N.V. 340/kg.d.w. <u>Coccidia</u> oocysts, numerous <u>Capillaria</u> sp. eggs (shell with shallow pits), V. 340/kg d w. <u>Ascaridia</u> -like eggs, V. 1,700/kg.d.w. <u>Ascaridia</u> -like eggs, N.V. 10,000/kg d.w.
Drying Bed	<u>Coccidia</u> oocysts, probably N.V., 2 or more types, numerous <u>Capillaria</u> sp. eggs (shell with striations), V. 70/kg.d.w. <u>Capillaria</u> sp. eggs (shell with striations), N.V. 130/kg.d.w. <u>Capillaria</u> sp. eggs (shell with pits), N.V. 70/kg.d.w. <u>Ascaridia</u> -like eggs, V. 4,500/kg.d.w. <u>Ascaridia</u> -like eggs, N.V. 3,700/kg d.w.

(continued)

TABLE B-5. (continued)

<u>Plant Site</u>	<u>Parasites</u>
11. 1 ⁰ Sludge ¹	<u>Coccidia</u> oocysts, V. & N.V., numerous <u>Hymenolepis</u> sp. (not <u>H. diminuta</u>), V. 240/kg.d.w. <u>Entamoeba coli</u> -like cysts, N.V. 240/kg.d.w. <u>Ascaridia</u> -like eggs, V. 12,000/kg.d.w. <u>Ascaridia</u> -like eggs, N.V. 11,000/kg.d.w.
Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations), N.V. 70/kg.d.w. <u>Ascaridia</u> -like eggs, V. 470/kg.d.w. <u>Ascaridia</u> -like eggs, N.V. 10,000/kg d.w.
14. 1 ⁰ Sludge	<u>Entamoeba coli</u> -like cysts, N.V. 480/kg.d.w.
Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations), V. 80/kg d.w. <u>Capillaria</u> sp. eggs (shell with striations), N.V. 560/kg.d.w. <u>Capillaria</u> sp. eggs (shell with pits), N.V. 80/kg.d.w.
16. 1 ⁰ Sludge	<u>Hymenolepis nana</u> eggs, V. 330/kg.d.w. <u>Hymenolepis nana</u> eggs, N.V. 330/kg.d.w. <u>Capillaria</u> sp. eggs (shell with striations), V. 660/kg.d.w.
Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations), V. 170/kg.d.w. <u>Capillaria</u> sp. eggs (shell with pits), N.V. 40/kg.d.w. <u>Ascaridia</u> -like eggs, N.V. 40/kg d.w.
17. Contact Stabilized ¹	<u>Entamoeba coli</u> -like cysts, N.V. 700/kg.d.w. <u>Ascaridia</u> -like eggs, V. 1,100/kg.d.w.
Aerobic Digested ¹	<u>Ascaridia</u> -like eggs, V. 930/kg.d.w. <u>Ascaridia</u> -like eggs, N.V. 460/kg.d.w.
18. 1 ⁰ Sludge	<u>Hymenolepis nana</u> eggs, N.V. 160/kg.d.w. <u>Entamoeba coli</u> -like cysts, N.V. few
19. 1 ⁰ Sludge	<u>Capillaria</u> sp. eggs (shell with striations), V. 110/kg.d.w. <u>Entamoeba coli</u> -like cysts, N.V. 110/kg.d.w.
Anaerobic Digester	<u>Capillaria</u> sp. eggs (shell with striations), V. 80/kg.d.w. <u>Capillaria</u> sp. eggs (shell with pits, not like <u>C. hepatica</u>), V. 80/kg.d.w. <u>Capillaria</u> sp. eggs (shell with pits, like <u>C. hepatica</u>), V. 80/kg.d.w.

(continued)

TABLE B-5. (continued)

<u>Plant-Site</u>	<u>Parasites</u>
22. Activated Sludge ¹	<u>Entamoeba coli</u> -like cysts, V. 1,600/kg d.w. <u>Entamoeba coli</u> -like cysts, N.V. 1,600/kg.d.w.
Aerobic Digester	<u>Hymenolepis nana</u> eggs, N.V. 330/kg.d.w.
Centrifuged Aerobic	<u>Trichosomoides</u> -like eggs, V. 220/kg.d.w. <u>Ascaridia</u> -like eggs, V. 220/kg.d.w.
24. 1 ⁰ & Activated Sludge	<u>Hymenolepis nana</u> eggs, N.V. 270/kg.d.w. <u>Entamoeba coli</u> -like cysts, N.V. few
25. 1 ⁰ Sludge	<u>Giardia</u> sp. cysts, N.V. numerous <u>Entamoeba coli</u> -like cysts, N.V. few <u>Toxascaris</u> -like egg, V. 120/kg.d.w.
28. Contact Stabilized	<u>Entamoeba coli</u> -like cysts, N.V. few
29. Thickened Activated	<u>Hymenolepis nana</u> eggs, V. 210/kg.d.w. <u>Entamoeba coli</u> -like cysts, N.V. few
30. 1 ⁰ Sludge	<u>Entamoeba coli</u> -like cysts, N.V. few
31. 1 ⁰ Sludge	<u>Entamoeba coli</u> -like cysts, N.V. few
34. 1 ⁰ Sludge	<u>Entamoeba coli</u> -like cysts, N.V. few
37. 1 ⁰ Sludge	<u>Capillaria</u> sp. eggs (shell with fine pits), V. 710/kg.d.w.
Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations), V. 250/kg.d.w.
38. 1 ⁰ Sludge	<u>Entamoeba coli</u> -like cysts, N.V. few <u>Toxascaris</u> -like eggs, V. 570/kg.d.w.

V.= Viable

N.V.= Nonviable

¹ five gallons of sewage settled overnight, then decanted; sample taken from remaining sediment

TABLE B-6. SLUDGE CHARACTERISTICS OF SAMPLES COLLECTED IN
WINTER (FEBRUARY AND MARCH, 1978)

Plant Site	Total Solids mg/l (**mg/g)	Total Volatile Solids mg/l (**mg/g)	Percent Moisture Content	Chemical Oxygen Demand mg/g	Kjeldahl Nitrogen mg/g	pH	Oxidation Reduction Potential
1. 1 st Sludge Drying Bed	63,400 **457	37,400 **111	54	835 759	26 7	5.4	-238
2. 1 st Sludge Drying Bed	35,400 **794	23,400 **171	21	624 694	21 11	5.5	-250
3. Aerobic Digester ¹	22,800	19,000		914	54	6.3	-221
4. 1 st Sludge Drying Bed	41,600 **264	33,000 **121	74	1,049 1,595	36 25	5.7	-241
6. Activated Sludge ¹ Drying Bed	12,800 **554	8,600 **217	45	715 1,163	53 27	6.1	-214
7. Activated Sludge ¹ Drying Bed	10,200 **237	7,600 **79	76	1,101 1,186	60 26	6.4	-245
8. 1 st Sludge Drying Bed	54,600 **539	39,000 **238	46	915 1,353	32 28	5.3	-193
10. 1 st Sludge Drying Bed	29,800 **513	25,200 **240	49	1,161 986	44 29	5.8	-239
11. 1 st Sludge ¹ Drying Bed	82,000 **494	51,800 **101	51	859 636	29 15	5.8	19
13. 1 st & Activated Sludge ¹ Aerobic Digester Drying Bed	19,000 11,600 **929	12,400 9,200 **329	7	986 1,384 801	29 44 24	6.5 7.0	-276 -247
14. 1 st Sludge Drying Bed	21,000 **418	17,600 **142	58	1,066 1,050	27 47	5.8	-306
16. 1 st Sludge Drying Bed	30,200 **767	22,600 **228	22	993 587	26 19	5.3	-380
17. Contact Stabilized ¹ Aerobic Digester ¹	13,400 17,200	10,000 9,000		336 262	33 19	6.7 6.5	-187 -153

(continued)

TABLE B-6 (continued)

Plant Site	Total Solids mg/l (**mg/g)	Total Volatile Solids mg/l (**mg/g)	Percent Moisture Content	Chemical Oxygen Demand mg/g	Kjeldahl Nitrogen mg/g	pH	Oxidation Reduction Potential
18. 1 ^o Sludge Drying Bed	63,600 **608	44,000 **236	39	996 755	27 26	5.9	-262
19. 1 ^o Sludge Anaerobic Digester	90,600 119,400	32,400 55,000		490 200	13 17	6.0 5.5	-273 -272
21. 1 ^o Sludge Drying Bed	30,600 **716	20,600 **203	28	1,011 473	31 16	6.1	-257
22. Activated Sludge ¹ Aerobic Digested Vacuum Activated Centrifuged Aerobic	12,600 30,800 **127 **154	8,000 17,800 **82 **83	87 85	1,086 828 1,000 716	49 41 44 36	7.2 7.0 4.2	-214 -170
24. 1 ^o & Activated Sludge Drying Bed	36,600 **503	25,600 **261	50	1,118 1,050	40 29	6.1	-251
25. 1 ^o Sludge Thickened Activated Drying Bed	83,800 19,400 **749	54,400 14,000 **200	25	361 1,027 401	21 52 14	5.7 6.6	-262 -212
26. Imhoff Digester Drying Bed	67,600 **467	40,200 **245	53	884 830	31 30	6.5	-286
28. Contact Stabilized Drying Bed	16,200 **559	12,000 **237	44	1,312 582	55 33	7.0	-186
29. Thickened Activated Drying Bed	47,400 **454	35,600 **283	55	1,076 1,112	46 43	6.5	-237
30. 1 ^o Sludge Drying Bed	42,400 **586	23,600 **217	41	896 375	22 13	7.3	-178
31. 1 ^o Sludge Drying Bed	19,400 **508	13,400 **151	49	1,116 606	23 14	6.6	-305
34. 1 ^o Sludge Drying Bed	33,200 **623	23,000 **258	38	1,268 774	40 21	6.0	-186

(continued)

TABLE B-6 (continued)

Plant Site	Total Solids mg/l (**mg/g)	Total Volatile Solids mg/l (**mg/g)	Percent Moisture Content	Chemical Oxygen Demand mg/g	Ammonia Nitrogen mg/g	pH	Oxidation Reduction Potential
37. 1 ^o Sludge Drying Bed	48,600 **538	21,400 **107	46	481 370	12 12	6.6	-182
38. 1 ^o Sludge Drying Bed	35,400 **889	24,800 **375	11	1,058 680	33 21	6.0	-290

1 five gallons of sewage settled overnight, then decanted; sample taken from remaining sediment.

TABLE B-7. SPRING (MAY - JUNE 1978) EXAMINATION FOR PARASITES IN SLUDGES FROM MUNICIPAL TREATMENT PLANTS OF THE SOUTHERN UNITED STATES

Plant Site	Actual Size Sample Examined Grams dry weight	<i>Ascaris</i>		<i>T. trichiura</i>		<i>E. vulpis</i>		<i>Toxocara</i>		<i>H. diminutus</i>	
		v	nv	v	nv	v	nv	v	nv	v	nv
		eggs/kg ²		eggs/kg ²		eggs/kg ²		eggs/kg ²		eggs/kg ²	
1. 1 st Sludge Drying Bed	5.42 19.56	9,200 4,400	550 1,800	550 50	13,000 510	1,500 1,200	180 820	2,400 200	1,100 510	0 0	1,300 310
2. 1 st Sludge Drying Bed	2.32 12.48	1,300 400	1,700 2,300	0 0	0 240	860 560	0 960	860 0	860 640	0 0	450 0
3. Aerobic Digester ¹	2.83	3,900	350	350	0	0	350	4,900	1,100	0	0
4. 1 st Sludge Drying Bed	3.91 23.85	1,000 0	4,600 0	0 0	1,300 0	3,600 80	2,100 1,300	1,500 0	1,000 0	0 0	1,500 0
6. 1 st Activated Sludge ¹ Drying Bed	2.15 25.47	470 0	0 40	0 0	0 0	0 0	0 0	1,900 0	470 0	0 0	0 0
7. Activated Sludge ¹ Drying Bed	3.38 17.82	100,000 74,000	10,000 37,000	2,400 1,100	1,200 2,600	1,200 510	0 860	300 170	300 510	300 0	0 60
8. 1 st Sludge Drying Bed	4.16 21.75	25,000 90	3,400 140	0 0	240 0	960 90	0 140	3,400 140	480 90	960 0	5,300 0
10. 1 st Sludge Drying Bed	4.81 28.53	830 0	1,000 0	0 0	0 0	420 0	0 70	0 0	0 0	0 0	450 0
11. 1 st Sludge Drying Bed	3.77 14.04	800 0	0 290	0 0	0 150	530 70	0 150	530 0	530 70	0 0	800 0
13. 1 st Activated Sludge ¹ Drying Bed	1.45 16.02	690 0	0 0	0 0	0 0	1,400 0	0 0	1,400 0	0 0	0 0	0 0
15. 1 st Sludge Drying Bed	4.64 12.78	0 550	0 3,000	0 0	0 470	220 310	0 860	650 0	0 1,100	0 0	0 160
16. 1 st Sludge Drying Bed	3.78 27.63	8,500 0	3,700 0	0 0	0 0	0 0	0 40	530 0	530 0	0 0	6,500 0

(continued)

TABLE B-7 (continued)

Plant Site	Actual Size Sample Examined grams dry weight	<i>Ascaris</i> v eggs/kg ²		<i>T. trichiura</i> v eggs/kg ²		<i>T. vulpis</i> v eggs/kg ²		<i>Toxocara</i> v eggs/kg ²		<i>H. diminuta</i> v eggs/kg ²	
17. Rerated Sludge ¹	3.20	6,900	940	0	0	0	0	630	630	0	310
Thickened Aerobic Digested Sludge ¹	4.82	6,000	2,100	0	0	620	0	210	0	0	0
18. 1 st Sludge	5.34	19,000	190	7,500	3,200	1,900	190	560	0	0	190
Drying Bed	27.60	0	0	0	0	180	1,100	0	0	0	0
19. 1 st Sludge	13.96	9,700	720	1,900	860	1,200	440	290	0	0	2,500
Anaerobic Digester	10.45	21,000	14,200	1,200	3,900	2,000	1,000	100	1,500	0	770
21. 1 st Sludge	1.17	1,700	0	0	0	2,600	0	0	0	0	1,700
Anaerobic Digester	8.80	3,200	1,500	1,900	2,100	1,400	230	1,100	2,800	0	1,000
Drying Bed	28.71	0	0	0	0	0	30	0	0	0	0
22. Aerobic Digester ¹	2.98	8,400	0	0	1,300	340	0	670	0	0	2,500
Activated Sludge ¹	3.94	3,300	0	0	500	760	0	1,000	0	0	0
Vacuum Activated	4.02	4,700	250	500	250	500	0	750	0	0	250
24. 1 st Sludge	3.63	7,400	1,100	0	1,100	0	0	630	0	0	0
Drying Bed	17.52	5,800	5,800	110	230	110	60	170	60	0	0
25. 1 st Sludge	3.86	0	0	0	0	260	0	0	0	0	0
Thickened Aerobic Digester Sludge	1.91	520	0	0	570	520	0	0	0	0	0
Drying Bed	28.26	0	0	0	0	35	70	0	0	0	0
26. Imhoff Digester	5.14	2,200	780	0	190	970	190	560	190	0	0
Drying Bed	27.00	40	260	0	0	70	170	0	0	0	0
28. 1 st Contact Stabilized	2.98	340	0	0	1,350	670	350	4,700	670	0	0
Drying Bed	4.62	1,500	220	0	1,700	650	0	220	650	0	0
29. Thickened Activated Sludge	4.29	1,200	230	0	0	500	0	500	0	0	530
Drying Bed	27.93	0	0	0	0	40	70	0	0	0	0

(continued)

TABLE B-7. (continued)

Plant Site	Actual Size grams dry weight	<i>Ascaris</i>		<i>T. trichiura</i>		<i>T. vulpis</i>		<i>Toxocara</i>		<i>H. diminuta</i>	
		v	nv	v	nv	v	nv	v	nv	v	nv
		eggs/Kg ²		eggs/Kg ²		eggs/Kg ²		eggs/Kg ²		eggs/Kg ²	
30. 1 ⁰ Sludge	7.30	270	0	0	140	140	0	270	0	0	410
Drying Bed	28.20	0	0	0	0	0	0	0	0	0	0
31. 1 ⁰ Sludge	3.87	260	0	0	0	0	0	260	260	0	520
Drying Bed-3month	4.44	0	450	0	0	0	230	0	230	0	230
Drying Bed-1 year	21.84	0	50	0	0	0	50	0	0	0	50
34. 1 ⁰ Sludge	4.19	480	0	240	0	1,700	240	240	0	0	240
Drying Bed	27.96	0	0	0	40	180	220	0	0	0	0
37. 1 ⁰ Sludge	8.84	8,800	3,300	4,800	3,300	110	110	230	110	0	0
Drying Bed	24.06	80	13,000	40	42,000	40	330	0	210	0	40
38. 1 ⁰ Sludge	3.27	0	0	0	0	0	0	0	0	0	0
Drying Bed	27.63	0	40	0	0	40	40	0	0	0	0

v = VIABLE

nv= NONVIABLE

1 five gallons of sewage settled overnight, then decanted, sample taken from remaining sediment

2 eggs/kilograms dry weight of sample

TABLE B-8. MISCELLANEOUS PARASITES (NOT INCLUDED IN TABLE 1) FOUND IN SLUDGES FROM MUNICIPAL TREATMENT PLANTS (SPRING - MAY AND JUNE 1978)

<u>Plant Site</u>	<u>Parasites</u>
1. 1 st Sludge	<u>Entamoeba coli</u> - like cysts, N.V. Few <u>Giardia</u> sp. cysts, N.V. Few <u>Capillaria</u> sp. eggs (shell with striations) V. 185/kg.d.w
Drying Bed	<u>Trichosomoides</u> - like eggs, N.V. 50/kg.d.w. <u>Capillaria</u> sp. eggs (shell with striations) N.V. 50/kg.d.w
2. 1 st Sludge	<u>Entamoeba coli</u> - like cysts, N.V. Few
Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations) N.V. 240/kg.d.w. <u>Capillaria</u> sp. eggs (shell with pits) V. 160/kg.d.w <u>Capillaria</u> sp. eggs (shell with pits) N.V. 80/kg d.w.
3. Aerobic Digester 1	<u>Entamoeba coli</u> - like cysts N.V. Few
4. 1 st Sludge	<u>Entamoeba coli</u> - like cysts N.V. Few
8. 1 st Sludge	<u>Capillaria</u> sp. eggs (shell with striations) V. 1,200/kg d.w. <u>Capillaria</u> sp. eggs (shell with striations) N.V. 480/kg.d.w. <u>Trichosomoides</u> - like eggs, V. 480/kg.d.w. <u>Trichosomoides</u> - like eggs N.V. 480/kg.d.w. <u>Entamoeba coli</u> - like cysts, N.V. Few
10. 1 st Sludge	<u>Coccidia</u> oocysts N.V. Few <u>Ascaridia</u> - like eggs, N.V. 620/kg d.w. <u>Capillaria</u> sp. eggs (shell with striations) N.V. 210/kg d.w.
11. 1 st Sludge	<u>Ascaridia</u> - like eggs, V. 800/kg d.w <u>Ascaridia</u> - like eggs, N.V. 11,000/kg d.w <u>Coccidia</u> oocysts (small) N.V. Moderate nos
Drying Bed	<u>Ascaridia</u> - like eggs, N.V. 6,100/kg d.w <u>Coccidia</u> oocysts - Many
14. 1 st Sludge	<u>Giardia</u> sp. cysts N.V. - Numerous
Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations) N.V. 160/kg d.w

(continued)

TABLE B-8 (continued)

16.	1° Sludge	<u>Entamoeba coli</u> - like cysts, N.V. - Several <u>Giardia</u> sp. cysts, N.V. - Many <u>Capillaria</u> sp. eggs (shell with striations) V. 530/kg.d.w. <u>Capillaria</u> sp. eggs (shell with striations) N.V. 530/kg.d.w. <u>Capillaria</u> sp. eggs (shell with pits) V. 270/kg d.w. <u>Coccidia</u> oocysts V. - Several
17.	Reaerated Sludge ¹	<u>Ascaridia</u> - like eggs, V. 310/kg.d.w. <u>Ascaridia</u> - like eggs, N.V. 1,300/kg.d.w.
	Thickened Aerobic Digested Sludge ¹	<u>Ascaridia</u> - like eggs, V. 620/kg d.w. <u>Ascaridia</u> - like eggs, N.V. 830/kg d.w.
18.	1° Sludge	<u>Entamoeba coli</u> - like cysts, N.V. - Many
19.	1° Sludge	<u>Capillaria</u> sp. eggs (shell with striations) V. 70/kg d.w.
	Anaerobic Digester	<u>Capillaria</u> sp. eggs (shell with striations) V. 100/kg.d.w. <u>Capillaria</u> sp. eggs (shell with striations) N.V. 100/kg d.w.
21.	Anaerobic Digester	<u>Capillaria</u> sp. eggs (shell with striations) V. 460/kg d.w. <u>Capillaria</u> sp. eggs (shell with striations) N.V. 340/kg.d.w. <u>Capillaria</u> sp. eggs (shell with pits) V. 230/kg.d.w.
22.	Activated Sludge ¹	<u>Entamoeba coli</u> - like cysts N.V. - Few
	Vacuum Activated	<u>Capillaria</u> sp. eggs (shell with striations) V. 500/kg.d.w. <u>Entamoeba coli</u> - like cysts, N.V. - Few
24.	1° Sludge	<u>Coccidia</u> oocysts (large - <u>Isospora</u> <u>Felis</u>) N.V. 280/kg.d.w.
26.	Imhoff Digester	<u>Entamoeba coli</u> - like cysts, N.V. - Few
29.	Thickened Activated	<u>Entamoeba coli</u> - like cysts, N.V. - Few
34.	1° Sludge	<u>Capillaria</u> sp. eggs (shell with striations) V. 230/kg.d.w. <u>Giardia</u> sp. cysts, N.V. - Few <u>Entamoeba coli</u> - like cysts, N.V. - Few
	Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations) N.V. 220/kg.d.w.
37.	1° Sludge	<u>Capillaria</u> sp. eggs (shell with striations) V. 110/kg.d.w.
	Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations) N.V. 130/kg.d.w. <u>Capillaria</u> sp. eggs (shell with pits) N.V. 40/kg.d.w.

¹ Five gallons of sewage settled overnight, thus decanted, sample taken from remaining sediment

TABLE 3-9 SLUDGE CHARACTERISTICS OF SAMPLES COLLECTED IN
SPRING (MAY - JUNE 1978)

Plant Site	Total Solids mg/l (**mg/g)	Total Volatile Solids mg/l (**mg/g)	Percent Moisture Content
1. 1 ^o Sludge Drying Bed	54,160 **632	31,320 **127	35
2. 1 ^o Sludge Drying Bed	23,240 **416	15,320 **138	58
3. Aerobic Digested ¹	28,320	20,640	
4. 1 ^o Sludge Drying Bed	39,120 **795	28,880 **424	21
6. 1 ^o +Activated ¹ Drying Bed	21,480 **849	12,880 **330	15
7. Activated ¹ Drying Bed	33,760 **594	18,200 **322	41
8. 1 ^o Sludge Drying Bed	41,640 **725	25,320 **334	27
10. 1 ^o Sludge Drying Bed	48,120 **951	36,840 **377	5
11. 1 ^o Sludge Drying Bed	37,680 **468	28,120 **159	53
13. 1 ^o +Activated ¹ Drying Bed	14,520 **534	8,960 **181	47
14. 1 ^o Sludge Drying Bed	46,360 **426	34,400 **146	57
16. 1 ^o Sludge Drying Bed	37,760 **921	25,720 **317	8
17. Anaerobic sludge ¹ Thickened Aerobic Digested ¹	32,200 48,240	17,600 16,880	
18. 1 ^o Sludge Drying Bed	53,440 **920	31,360 **290	8
19. 1 ^o Sludge Anaerobic Digested	139,600 104,520	35,760 39,480	
21. 1 ^o Sludge Anaerobic Digested Drying Bed	11,720 88,000 **957	8,040 37,760 **248	4

(continued)

TABLE B-9 (continued)

Plant Site	Total Solids mg/l (**mg/g)	Total Volatile Solids mg/l (**mg/g)	Percent Moisture Content
22. Activated Sludge ¹ Aerobic Digested Volume-Activated	39,440 29,300 **134	23,360 14,720 **83	87
24. 1 ^o Sludge Drying Bed	36,360 584	25,440 218	42
25. 1 ^o Sludge Thickened Aerobic Digested Drying Bed	38,600 19,080 **942	26,560 10,600 **336	6
26. Imhoff Digester Drying Bed	51,440 **900	27,240 **350	10
28. 1 ^o + Contact Stabilized Drying Bed	29,760 **154	20,240 **97	85
29. Thickened-Activated Drying Bed	42,920 **931	26,680 **536	7
30. 1 ^o Sludge Drying Bed	72,960 **940	32,960 **100	60
31. 1 ^o Sludge Drying Bed-3 mos. Drying Bed-1 year	38,760 **148 **728	26,120 **67 **208	85 27
34. 1 ^o Sludge Drying Bed	41,920 **932	66,720 **310	7
37. 1 ^o Sludge Drying Bed	38,360 **802	37,040 **132	20
38. 1 ^o Sludge Drying Bed	32,680 **921	24,640 **391	8

Five gallons of sewage settled overnight.
Samples taken from the sediment.

TABLE B-10. SUMMER (AUGUST - SEPTEMBER 1978) EXAMINATION FOR PARASITES IN SLUDGES FROM MUNICIPAL TREATMENT PLANTS OF THE SOUTHERN UNITED STATES

Plant Site	Actual Size Sample Examined grams dry weight	<i>Ascaris</i> v eggs/kg ²	<i>nv</i> eggs/kg ²	<i>T. trichiura</i> v eggs/kg ²	<i>nv</i> eggs/kg ²	<i>I. vulpis</i> v eggs/kg ²	<i>nv</i> eggs/kg ²	<i>Toxocara</i> v eggs/kg ²	<i>nv</i> eggs/kg ²	<i>H. diminuta</i> v eggs/kg ²	<i>nv</i> eggs/kg ²
1. 1 st Sludge Drying Bed	5.58 17.17	10,000 5,400	540 23,200	0 60	0 820	1,100 640	180 1,500	2,200 0	900 1,900	0 0	2,500 100
2. 1 st Sludge Drying Bed	4.84 21.89	1,700 0	1,000 90	0 0	0 0	1,200 0	0 90	830 50	210 0	0 0	630 0
3. Aerobic Digester 1	2.73	9,900	2,200	370	0	370	370	5,900	2,600	0	0
4. 1 st Sludge Drying Bed	4.87 4.95	210 0	2,700 0	0 0	410 3,600	3,900 0	410 0	0 0	410 0	0 0	1,000 0
6. 1 st Activated Sludge ^k Drying Bed	2.40 27.36	1,300 0	830 0	0 0	0 0	420 0	0 0	430 0	0 0	0 0	830 0
7. Contact Stabilized Sludge ^k Drying Bed	0.82 19.30	69,000 28,000	9,700 36,000	3,600 1,200	0 1,000	0 470	0 730	0 50	0 420	0 0	0 0
8. 1 st Sludge Drying Bed	1.83 7.47	32,000 18,000	10,000 7,800	0 0	0 0	0 270	0 670	1,600 400	0 670	0 0	2,700 5,300
10. 1 st Sludge Drying Bed	2.80 11.16	3,600 630	2,500 2,000	0 0	0 90	1,400 630	0 810	1,900 0	730 0	0 0	5,300 130
11. 1 st Sludge Drying Bed	5.18 12.87	580 80	0 930	190 0	0 80	580 0	0 310	0 0	0 310	0 0	0 30
13. 1 st Activated Sludge Aerobically Digested Sludge ^k	1.86 0.86	1,100 1,200	0 0	0 0	0 0	540 1,200	0 1,200	2,700 0	0 1,200	0 0	0 0
15. 1 st Sludge Drying Bed	4.85 27.82	10 0	0 470	0 0	0 0	210 0	0 290	1,000 0	410 0	0 0	410 0

(continued)

TABLE B-10. (continued)

Plant Site	Actual Size Sample Examined grams dry weight	<i>Ascaris</i>		<i>T. trichiura</i>		<i>I. vulpis</i>		<i>Toxocara</i>		<i>H. diminuta</i>	
		v	nv	v	nv	v	nv	v	nv	v	nv
		eggs/kg ²		eggs/kg ²		eggs/kg ²		eggs/kg ²		eggs/kg ²	
29. Thickened Activated Sludge	1.92	0	0	0	0	1,000	0	1,600	0	0	500
Drying Bed	27.87	0	0	0	0	0	0	0	0	0	0
30. 1 st Sludge	3.35	900	0	0	300	300	0	300	0	0	600
Drying Bed	26.19	0	0	0	0	0	0	0	0	0	0
31. 1 st Sludge	2.25	890	0	0	440	0	0	440	0	0	890
Drying Bed	28.05	0	0	0	0	0	0	0	0	0	0
34. 1 st Sludge	4.44	2,900	0	0	450	0	0	1,100	0	0	900
Drying Bed	9.21	330	870	0	220	110	540	0	110	0	220
37. 1 st Sludge	20.50	15,000	240	16,000	4,100	290	50	200	50	0	750
Drying Bed	23.13	0	0	0	860	0	90	0	0	0	0
38. 1 st Sludge	3.20	310	0	0	630	630	0	130	0	0	0
Drying Bed	28.38	0	0	0	0	0	50	0	0	0	0

v = VIABLE

nv = NONVIABLE

1 five gallons of sewage settled overnight, then decanted, sample taken from remaining sediment

2 eggs/kilogram dry weight of sample

3 Rain caused flushing of clarifier; thus, five gallons of sewage settled overnight, then decanted, sample taken from remaining sediment.

TABLE B-11. MISCELLANEOUS PARASITES (NOT INCLUDED IN TABLE 1) FOUND IN SLUDGES FROM
MUNICIPAL TREATMENT PLANTS (SUMMER - AUGUST AND SEPTEMBER, 1978)

<u>Plant-Site</u>		<u>Parasites</u>
1.	1 Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations) N.V. 180/kg d.w
4.	1° Sludge	<u>Entamoeba coli</u> -like cyst N.V. - Few <u>Coccidia</u> oocysts (large - <u>Isoospora felis</u>) V. - few
7.	Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations) N.V. 50/kg. d w
8.	1° Sludge	<u>Ascaridia</u> - like eggs, V. 1,100/kg. d.w. <u>Entamoeba coli</u> - like cysts, N.V. - Many <u>Trichosomoides</u> - like eggs, N.V. 550/kg. d w
	Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations) V. 400/kg. d w. <u>Capillaria</u> sp. eggs (shell with striations) N.V. 400/kg.d w. <u>Hymenolepis nana</u> - like eggs, N.V. 270/kg.d w
10.	1° Sludge	<u>Ascaridia</u> - like eggs, N.V. 2,900/kg.d w. <u>Trichosomoides</u> - like eggs, N.V. 360/kg.d w <u>Coccidia</u> oocysts, N.V. - few <u>Entamoeba coli</u> - like cysts, N.V. - few
	Drying Bed	<u>Ascaridia</u> - like eggs, N.V. 900/kg. d.w
11.	1° Sludge	<u>Ascaridia</u> - like eggs, V. 24,000/kg.d w. <u>Coccidia</u> oocysts (small) N.V. - few
	Drying Bed	<u>Ascaridia</u> - like eggs, N.V. 21,000/kg.d.w
17.	Reaeration Sludge	<u>Ascaridia</u> - like eggs, V. 3,500/kg d w
	Thickened Aerobic Digested Sludge	<u>Ascaridia</u> - like eggs, V. 12,000/kg d w <u>Ascaridia</u> - like eggs, N.V. 1,600/kg.d w
18.	1° Sludge	<u>Capillaria</u> sp. eggs (shell with pits) V. 210/kg d w. <u>Entamoeba coli</u> - like cysts, N.V. - Many
19.	Anaerobic Digested	<u>Capillaria</u> sp. eggs (shell with striations) V. 120/kg. d w

(continued)

TABLE B-11. (continued)

21.	Anaerobic Digested	<u>Capillaria</u> sp. eggs (shell with striations) V. 330/kg.d.w. <u>Capillaria</u> sp. eggs (shell with pits) V. 490/kg.d.w. <u>Capillaria</u> sp. eggs (shell with pits) N.V. 160/kg.d.w.
22.	Activated Sludge ¹	<u>Trichosomoides</u> - like eggs, V. 770/kg.d.w.
	Aerobic Digested	<u>Trichosomoides</u> - like eggs, V. 450/kg.d.w.
	Vacuum Activated	<u>Ascaridia</u> - like eggs, V. 590/kg.d.w.
	Centrifuged Aerobic	<u>Capillaria</u> sp. eggs (shell with pits) N.V. 170/kg.d.w.
24.	1° Sludge	<u>Entamoeba coli</u> - like cysts N.V.
25.	1° Sludge	<u>Toxascaris Leonina</u> eggs, V. 190/kg.d.w.
29.	Thickened Activated	<u>Entamoeba coli</u> - like cysts, N.V. - Few <u>Capillaria</u> sp. eggs (shell with fine pits) V. 520/kg.d.w.
30.	1° Sludge	<u>Entamoeba coli</u> - like cysts, N.V. - Few <u>Giardia</u> sp. cysts, N.V. - Few
31.	1° Sludge	<u>Entamoeba coli</u> - like cysts, N.V. - Few <u>Giardia</u> sp. cysts, N.V. - Few <u>Capillaria</u> sp. eggs (shells with striations) V. 450/kg.d.w.
34.	1° Sludge	<u>Entamoeba coli</u> - like cysts, N.V. - Few <u>Giardia</u> sp. cysts, N.V. - Few <u>Capillaria</u> sp. eggs (shell with striations) V. 230/kg.d.w.
	Drying Bed	<u>Capillaria</u> sp. eggs (shell with striations) N.V. 220/kg.d.w.
47.	1° Sludge	<u>Entamoeba coli</u> - like cysts, N.V. - Many <u>Giardia</u> sp. cysts, N.V. - Many <u>Capillaria</u> sp. eggs (shell with striations) V. 200/kg.d.w.
38.	1° Sludge	<u>Entamoeba coli</u> - like cysts, N.V. - Few <u>Giardia</u> sp. cysts, N.V. - Few

¹ Five gallons of sewage settled overnight, thus decanted; sample taken from remaining sediment

TABLE B-12. SLUDGE CHARACTERISTICS OF SAMPLES COLLECTED IN
SUMMER (AUGUST - SEPTEMBER, 1973)

Plant Site		Total Solids mg/l (**mg/g)	Total Volatile Solids mg/l (**mg/g)	Percent Moisture Content
1.	1 ^o Sludge Drying Bed	53,800 **572	30,600 **166	43
2.	1 ^o Sludge Drying Bed	48,400 **730	33,080 **205	27
3.	Aerobic Digested ¹	27,280	19,960	
4.	1 ^o Sludge Drying Bed	48,680 **817	35,120 **415	18
6.	1 ^o Activated ¹ Drying Bed	24,740 **912	13,520 **347	9
7.	Contact Stabilized ¹ Drying Bed	8,280 **643	6,240 **329	37
8.	1 ^o Sludge Drying Bed	18,320 **249	14,200 **107	75
10.	1 ^o Sludge Drying Bed	27,960 **372	20,680 **194	63
11.	1 ^o Sludge Drying Bed	51,760 **429	38,640 **133	57
13.	1 ^o Activated ¹ Aerobic Digested ¹	18,640 8,600	11,360 4,960	
14.	1 ^o Sludge Drying Bed	48,360 **927	35,160 **262	7.3
16.	1 ^o Sludge Drying Bed	16,000 **915	11,560 **352	9
17.	Reaerated Sludge ¹ Thickened Aerobic Digested*	23,080 25,800	14,000 13,040	
18.	1 ^o Sludge Drying Bed	44,160 **823	28,360 **255	18
19.	1 ^o Sludge ¹ Anaerobic Digested	4,360 83,880	1,360 34,840	
21.	1 ^o Sludge Anaerobic Digested	13,240 61,320	3,120 22,520	

(continued)

TABLE 3-12. (continued)

Plant Size	Total Solids mg/l (**mg/g)	Total Volatile Solids mg/l (**mg/g)	Percent Moisture Content
22. Activated Sludge ¹	25,040	10,800	
Aerobic Digested ¹	22,160	11,140	
Volume-Activated	**170	**164	83
Centrifuged-Aerobic	**192	**72	81
24. 1 ^o Sludge	22,000	15,360	
Drying Bed	**611	**248	39
25. 1 ^o Sludge	51,680	32,360	
Thickened Aerobic			
Digested	11,680	7,000	
Drying Bed	**855	**298	15
26. Imhoff Digester	64,440	34,880	
Drying Bed	797 ¹ *	335	20
28. 1 ^o Contact Stabilized	13,280	9,200	
Drying Bed	**802	**389	20
27. Thickened Activated	19,240	15,280	
Drying Bed	**929	**473	7
30. 1 ^o Sludge	33,480	23,360	
Drying Bed	**873	**212	13
31. 1 ^o Sludge	22,520	15,520	
Drying Bed	**935	**321	7
34. 1 ^o Sludge	44,400	29,400	
Drying Bed	307	120	69
37. 1 ^o Sludge	205,040	56,520	
Drying Bed	**771	**107	23
38. 1 ^o Sludge	32,000	20,280	
Drying Bed	**946	**448	5

¹ Five gallons of sewage settled overnight. Samples taken from the sediment.

TABLE B-13. SLUDGE CHARACTERISTICS OF SAMPLES COLLECTED IN
FALL (NOVEMBER AND DECEMBER, 1977)

Plant Classification	Size MGD	Population Served	Age Years	Types of Wastes	Treatment Process of Sludge	Ultimate Disposal
1. TF-AnSD	8.5	50,000	35	Domestic, 1 plating co.	1 ⁰ clarifier → anaerobic digester (18 days) → vacuum filtration → drying beds	Landfill
2. Pure Oxygen	16	120,000	19	Domestic	1 ⁰ & AS clarifier → anaerobic digester (30 days) → drying beds	Mixed with soil for commercial sale
3. AS-AerD	1.1	10,000	25	Domestic	1 ⁰ & TF clarifier → aerobic digester (15 days) → pump trucks	Truck garden
4. TF-AnSD	0.5	6,600	15	Domestic	1 ⁰ & TF clarifier → 1 ⁰ anaerobic digester → 2 ⁰ anaerobic digester (30 days total) → drying beds	Landfill
5. TF-AerD	0.4	5,300	20	Domestic	1 ⁰ & 2 ⁰ clarifier → aerobic digester → drying beds	Landfill, local gardens
6. AS-AerD	0.4	5,000	8	Domestic	Influent → AS → clarifier → aerobic digester → drying beds	Landfill
7. AS-AerD	2.5	3,350	7	60% Industrial (swine, cattle peanut-oil mill)	Influent → AS → clarifier → thickener → aerobic digester (14 days) → drying beds	Landfill
8. TF-AnSD	0.3	5,000(?)	24	Domestic	1 ⁰ & TF clarifier → anaerobic digester (30 days) → drying beds	Farmers, gardens, lawns, worm farms
9. TF-AnSD	0.9	4,500	20	5% rubber industry waste	1 ⁰ clarifier → anaerobic digester → drying beds	City landfill
10. AS-TF-AnSD	10	100,000	21	70% industrial (meat packing, 2 milk co., poultry plant, bread co.)	TF clarifier → AS → AS clarifier & 1 ⁰ clarifier → 1 ⁰ anaerobic digester → 2 ⁰ anaerobic digester (45 days total) → drying beds	Gardens, lawns School-yards
11. TF-AnSD	2.5	14,000	15	30% poultry waste	1 ⁰ clarifier → anaerobic digester (40 days) → drying beds	Lawns, gardens, church & school- yards, parks, fields

(continued)

TABLE B-13. (continued)

Plant Classification	Size MGD	Population Served	Age Years	Types of Wastes	Treatment Process of Sludge	Ultimate Disposal
12. TF-AerD	3.1	15,000	11	20% textile waste	1 ⁰ & 2 ⁰ clarifiers + 1 ⁰ aerobic digester + 2 ⁰ aerobic digester + drying beds	School-yards, pastures, gardens, parks
13. AS-AerD	3.2	25,000	6	Domestic	Influent + AS + clarifier + aerobic digester (5 days) + lagoon or drying beds	drying bed only when need landfill
14. 1 ⁰ clarified-AnSD	30	301,000	20	All kinds	1 ⁰ clarifier + 1 ⁰ anaerobic digester + 2 ⁰ anaerobic digester + drying bed or vacuum filtration + flash drier	Nurseries, schools, gardens, churches
15. AS-AnSD	10	80,000	3	25% meat packing, chicken processing, herbicide manufact.	1 ⁰ clarifier + anaerobic digester + lagoon	lagoon
16. AS-AnSD	6.5	50,000	19	2 small slaughter houses, plant that makes ceiling tile (0.3 MGD)	1 ⁰ & AS clarifier + anaerobic digester (28 days) + drying beds	Anyone that wants it
17. AS-AerD	30	154,000	2	all kinds	Influent + contact stabilization + clarifier + aerobic digester + (28 days) + lagoon (3 years)	Sanitary landfill
18. AS-AnSD	5	45,000	19	2 pepper factories	1 ⁰ clarifier + AS + clarifier + 1 ⁰ anaerobic digester + 2 ⁰ anaerobic digester (21 days total) + drying beds	Gardens, farms
19. TF-AnSD	1	13,000	13	rice mill	1 ⁰ & TF clarifier + anaerobic digester + pump truck	Pastures in liquid form
20. AS-AnSD	7	50,000	7	milk processing	Digesters being repaired at this time, sludge from 1 ⁰ clarifier going to canal or to farms	Pastures
21. AS-AnSD	1.3	13,000	21	Domestic	1 ⁰ clarifier + anaerobic digester (45 days) + pump truck or drying bed	Wet: pastures Dry: gardens

(continued)

TABLE B-13. (continued)

Plant Classification	Size MGD	Population Served	Age Years	Types of Waste	Treatment Process of Sludge	Ultimate Disposal
22. AS-AerD	80	460,000	3	30%: Brewery wastes, coffee processing, poultry and slaughter houses	Influent → AS → clarifier → aerobic digester → centrifuge → landfill, or clarifier → vacuum filter (lime added) → landfill, or clarifier → vacuum filter → flash dryer → fertilizer	Landfill for centrifuged & vacuum filtered, flash dried to orange groves
23. TF-AnSD	0.7	6,000	4	Meat packing plant	1 st & 2 nd clarifier → aerobic digester → drying beds	Sanitary landfill
24. AS-AnSD	94	600,000	14	Meat packing, food processing, brewery	1 st & AS clarifier → 1 st anaerobic digester → 2 nd anaerobic digester (28 days total) → drying beds	Sold for fertilizer
25. AS-AerD-AnSD	7	230,000	7	Domestic	1 st clarifier → 1 st anaerobic digester → 2 nd anaerobic digester (60 days total) → drying bed, and Effluent from 1 st clarifier → AS → clarifier → aerobic digester (60 days) → thickener → spray on fields	Dry city uses for trees Wet: Pastures
26. Imhoff	4.5	17,000	18	2 slaughter houses	Influent → Imhoff digester (40 days) → drying beds	Gardens
27. TF-AnSD	1.3	18,860	23	Domestic	1 st clarifier → anaerobic digester → drying beds	Landfill
28. Contact S-AerD	2.5	45,000	9	Domestic	1 st clarifier → contact stabilization → aerobic digester → drying bed	Pastures & Gardens
29. AS-AerD	3.0	8,696	3	Packing houses, plastic factory	Influent → AS → clarifier → thickener → aerobic digester (15 days) → drying beds	Pastures & Gardens
30. TF-AnSD	30	300,000	50	Slaughter houses	1 st clarifier → anaerobic digester (15 days) → lagoon (2 years)	Road grass & Gardens
31. AS-AnSD	35	375,000	22	Brewery, packing house	1 st & AS clarifier → anaerobic digester (30 days) → drying beds	Golf courses & parks

(continued)

TABLE B-13. (continued)

Plant Classification	Size MGD	Population Served	Age Years	Types of Wastes	Treatment Process of Sludge	Ultimate Disposal
32. 1 ^o clarifier-AerD or TF-AnSD	2.0	17,000	4	Paint factory & slaughter house	1 ^o & 2 ^o clarifier + macrobic digester + drying bed, or aerobic digester + drying bed.	Cell course
33. TF-AnSD	3.0	35,000	7	Margarine Co., meat packing, aluminum	1 ^o clarifier + anaerobic digester + drying bed	Yards & garden
34. TF-AnSD	6.0	58,000	11	Slaughter house, metal processing, oil	1 ^o clarifier + anaerobic digester (30 days) + drying beds	Landfill
35. TF-AnSD	?	23,000	?	not known	1 ^o clarifier + anaerobic digester + drying bed	Not known
36. TF-AnSD	?	3,200	11	Domestic	1 ^o & 2 ^o clarifier + anaerobic digester + drying beds	Operator uses on garden
37. TF-AnSD	?	5,400	16	Domestic	1 ^o clarifier + anaerobic digester + drying beds	Gardens
38. TF- & AS-AnSD	17	180,000	?	Cattle lot runoff, poultry, cotton oil mill	1 ^o & TF & AS clarifier + anaerobic digester (15 days) + drying beds	Dumpground, very little for private use

ABBREVIATIONS USED.

MGD. Millions of gallons per day

TF. Trickling Filter

AS: Activated Sludge

AnSD: Anaerobic Digester

AerD. Aerobic Digester

Contact S: Contact Stabilization

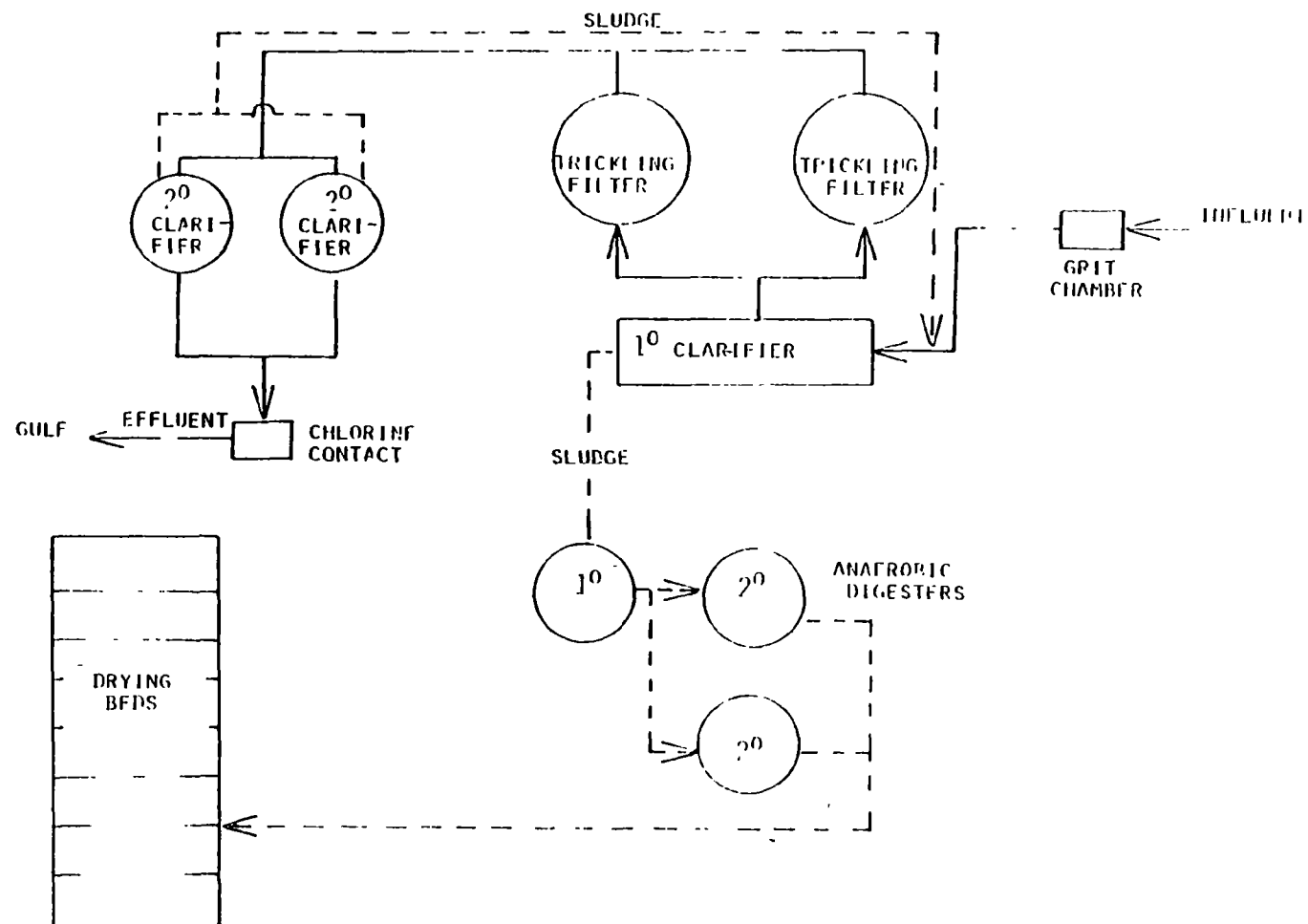


Figure B-1 Sewage Treatment Plant #1

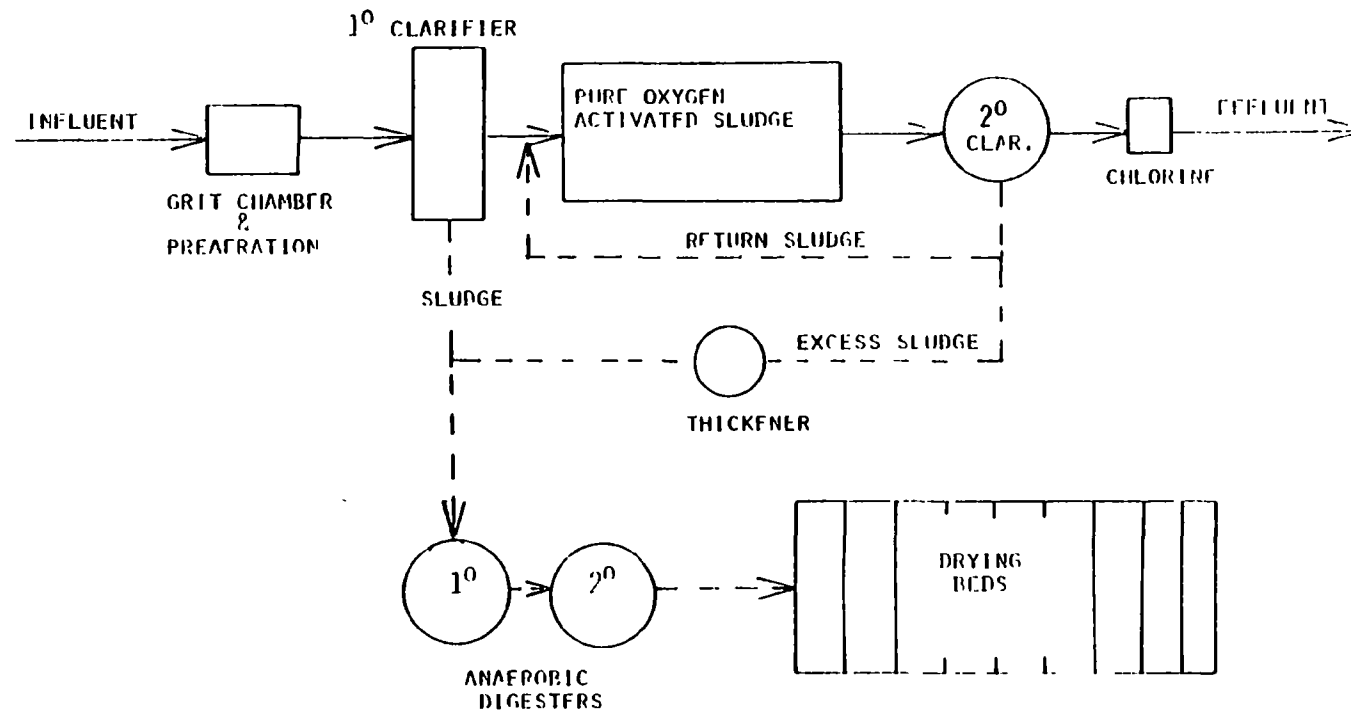


Figure B-2. Sewage Treatment Plant #2.

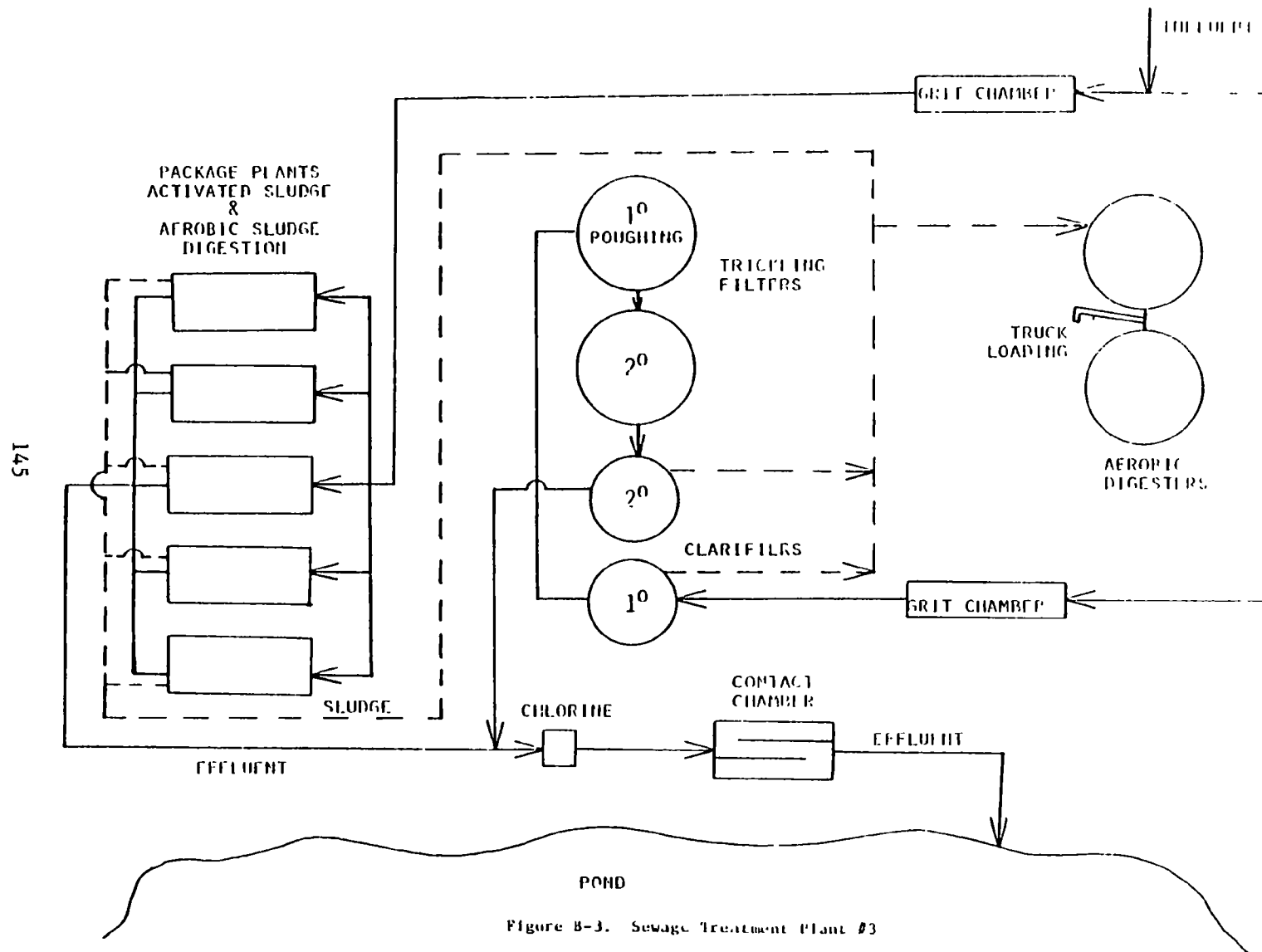


Figure B-3. Sewage Treatment Plant #3

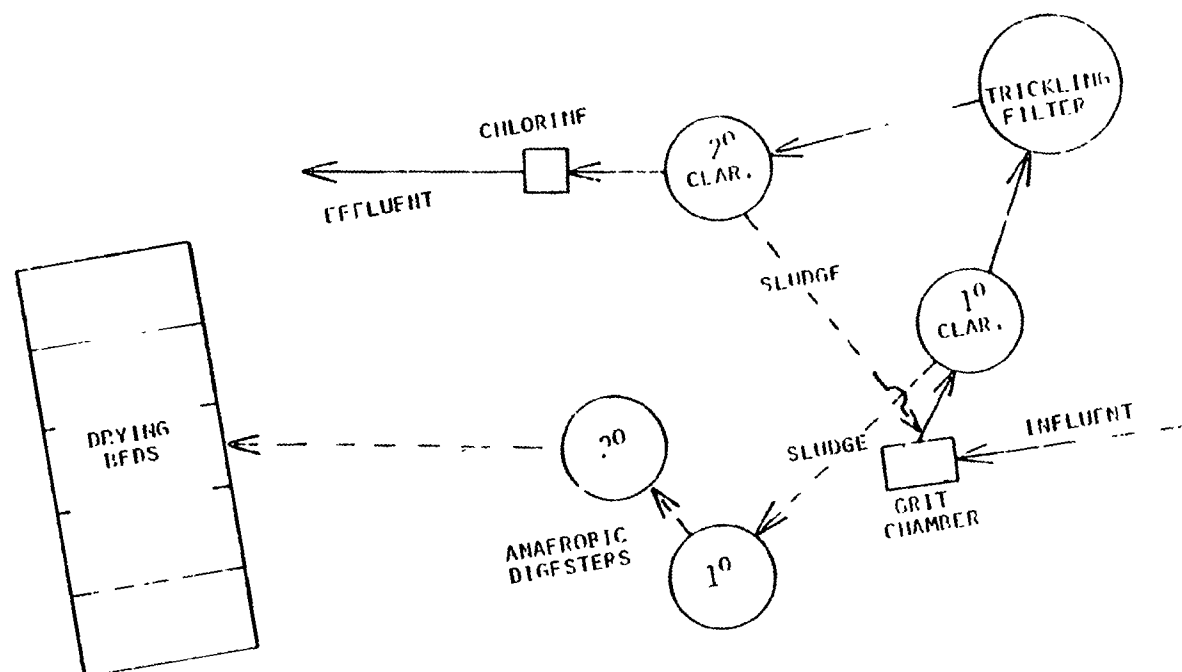


Figure B-4. Sewage Treatment Plant #4

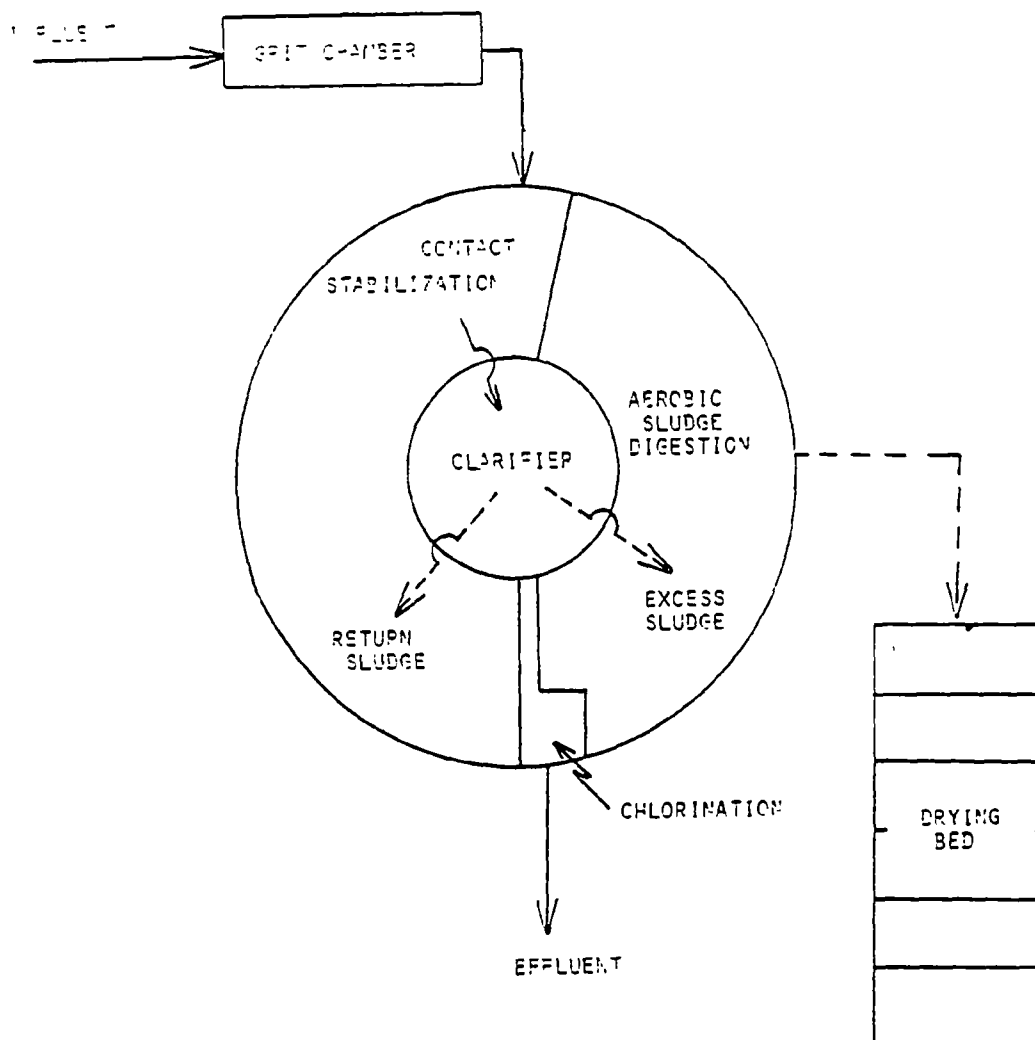


Figure 3-5. Sewage Treatment Plant #6.

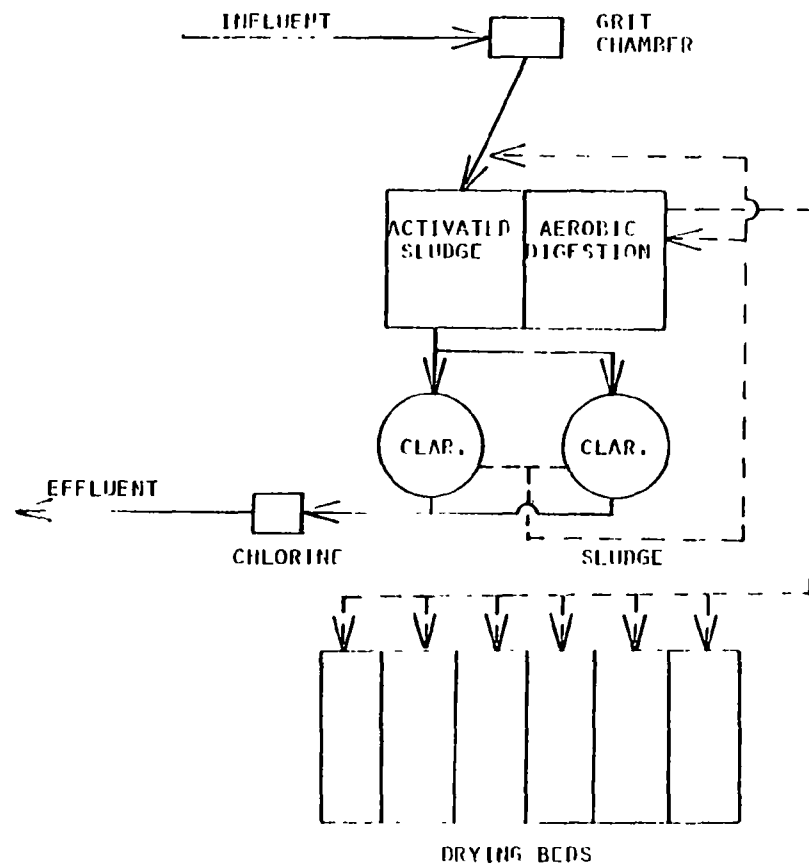


Figure B-6. Sewage Treatment Plant #1

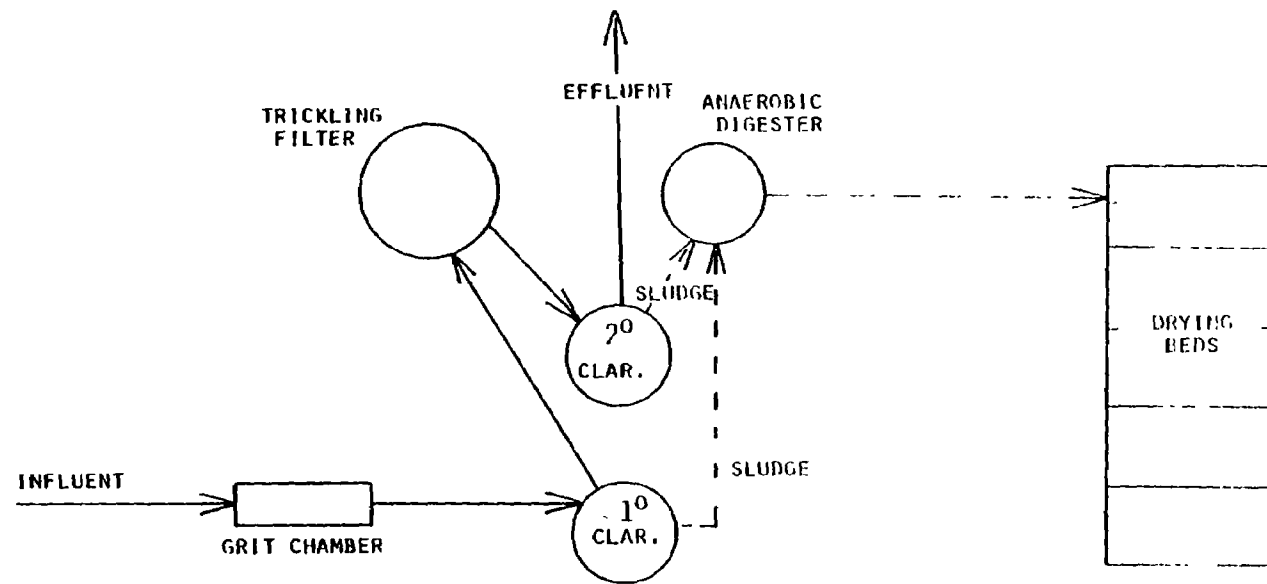


Figure B-7. Sewage Treatment Plant #8

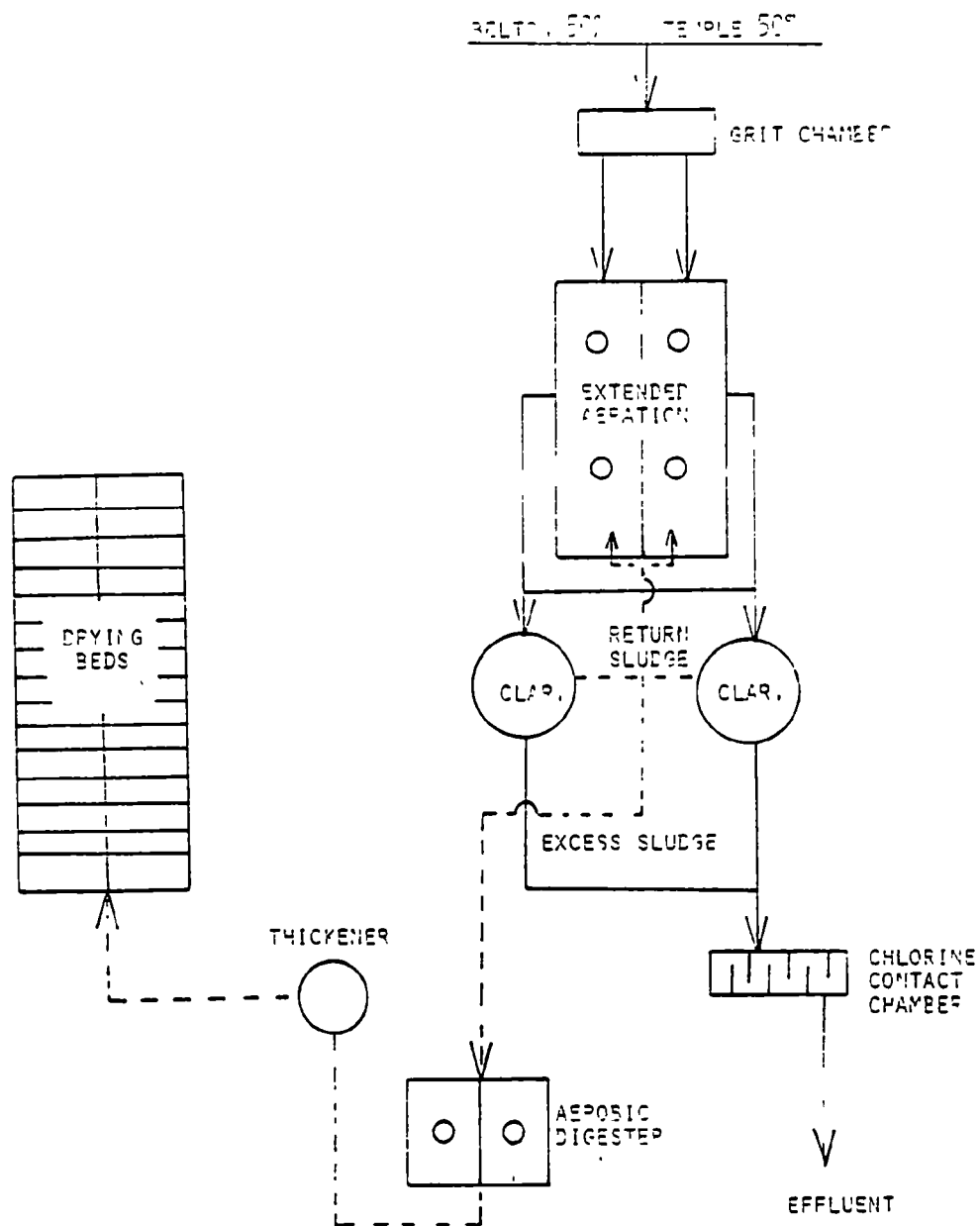


Figure B-8. Sewage Treatment Plant #9.

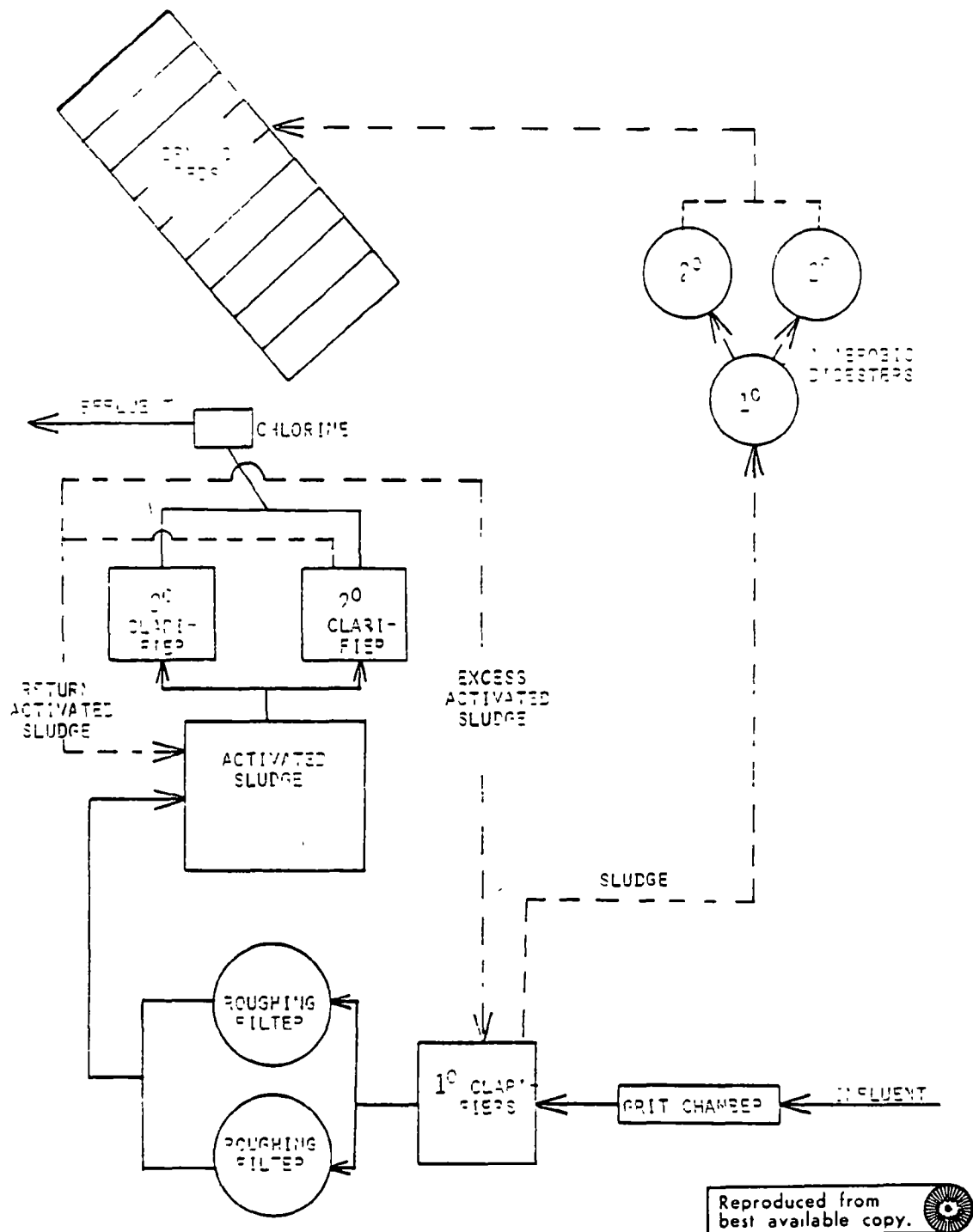


Figure 3-9. Sewage Treatment Plant #10.

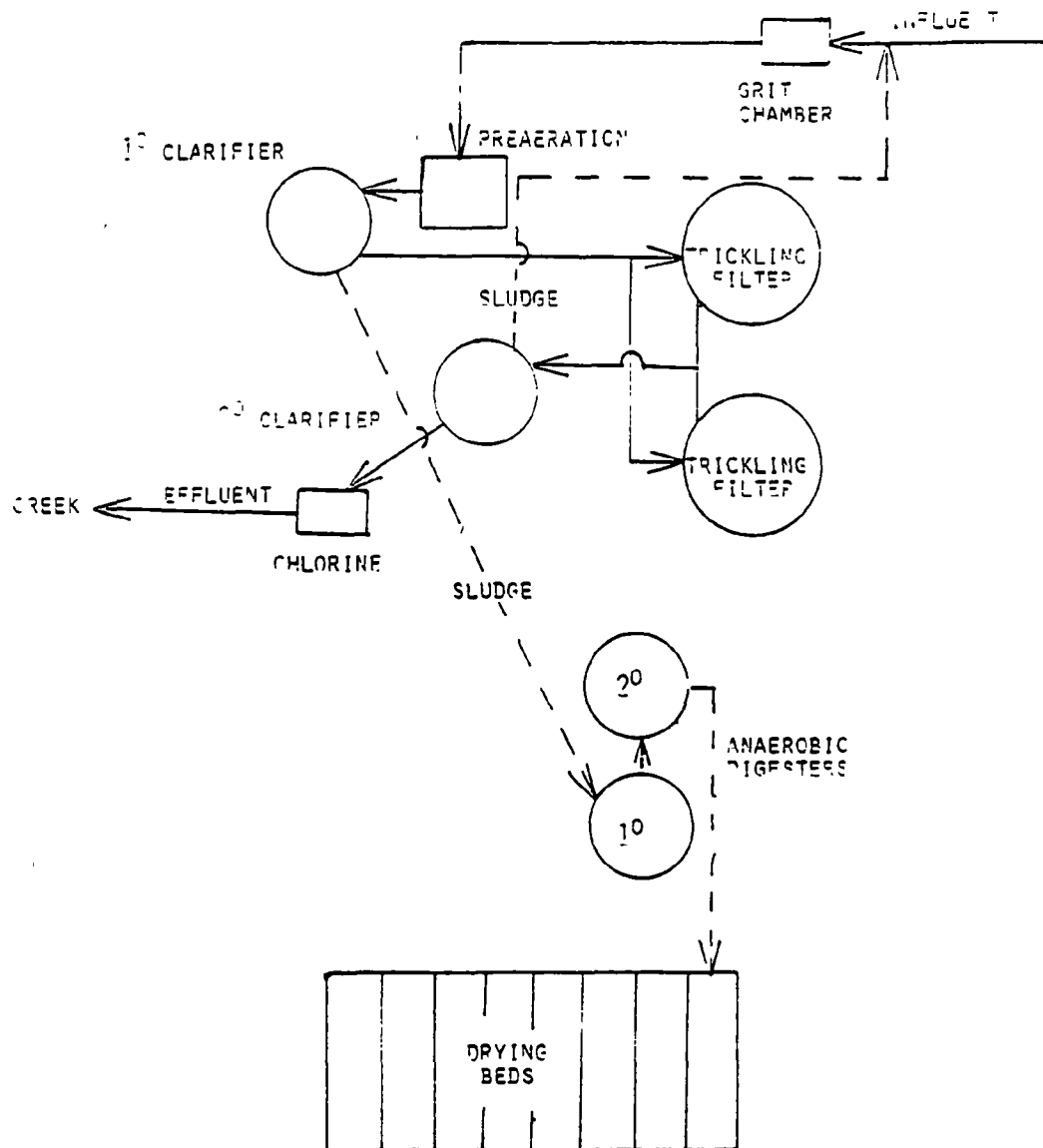


Figure B-10. Sewage Treatment Plant #11.

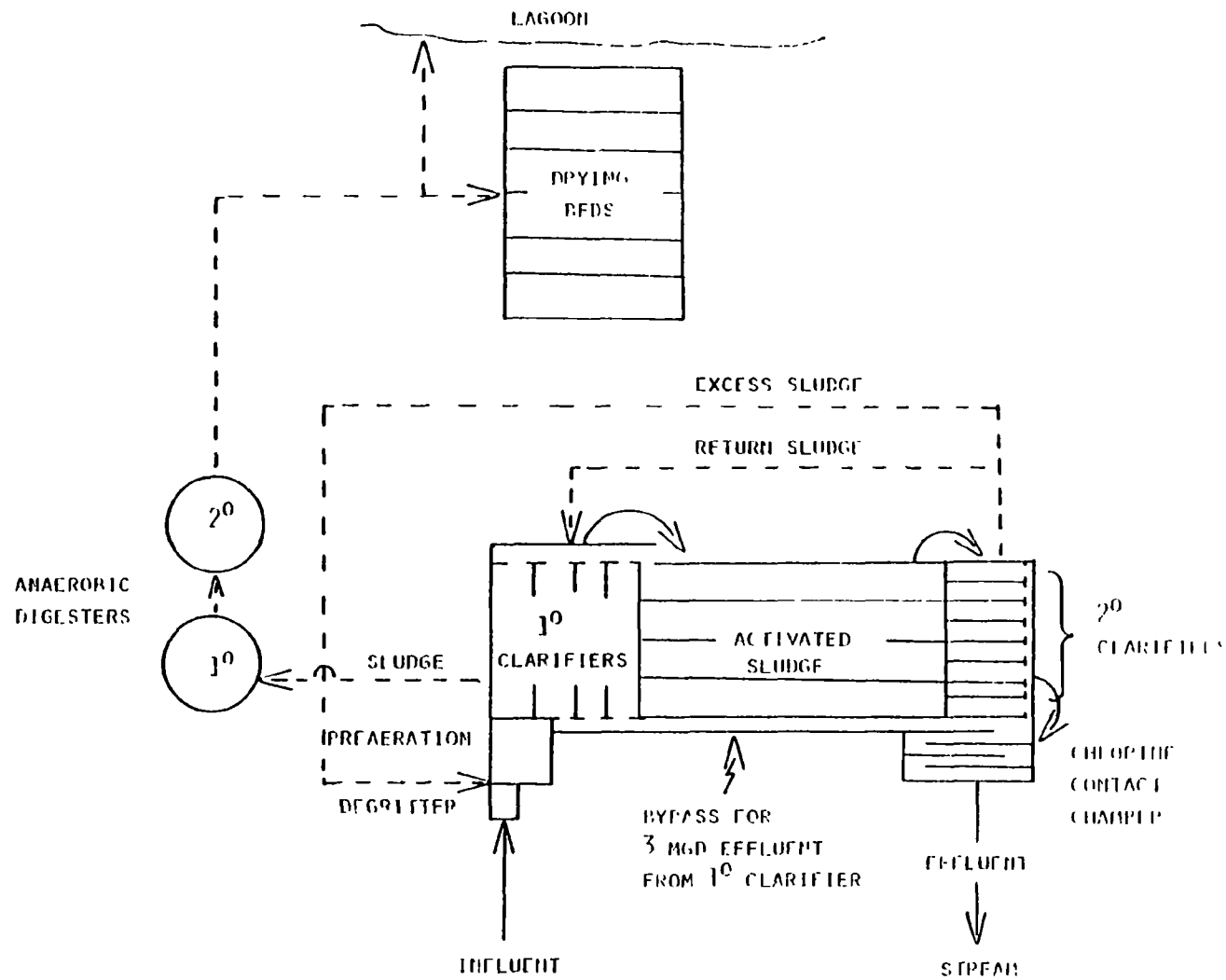


Figure B-11 Sewage Treatment Plant #16

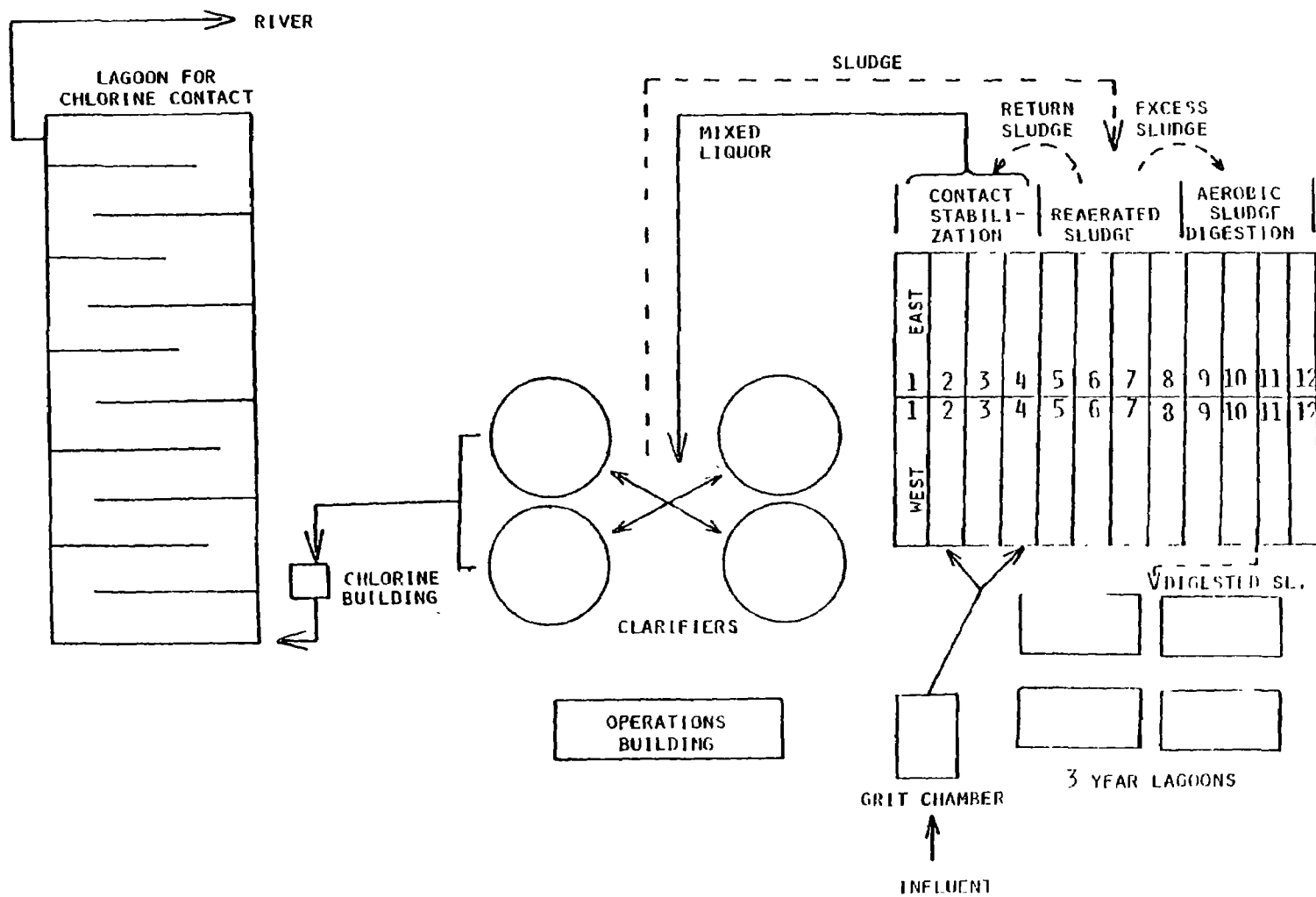


Figure 8-12 Sewage Treatment Plant #17

Figure B-13. Sewage Treatment Plant #18

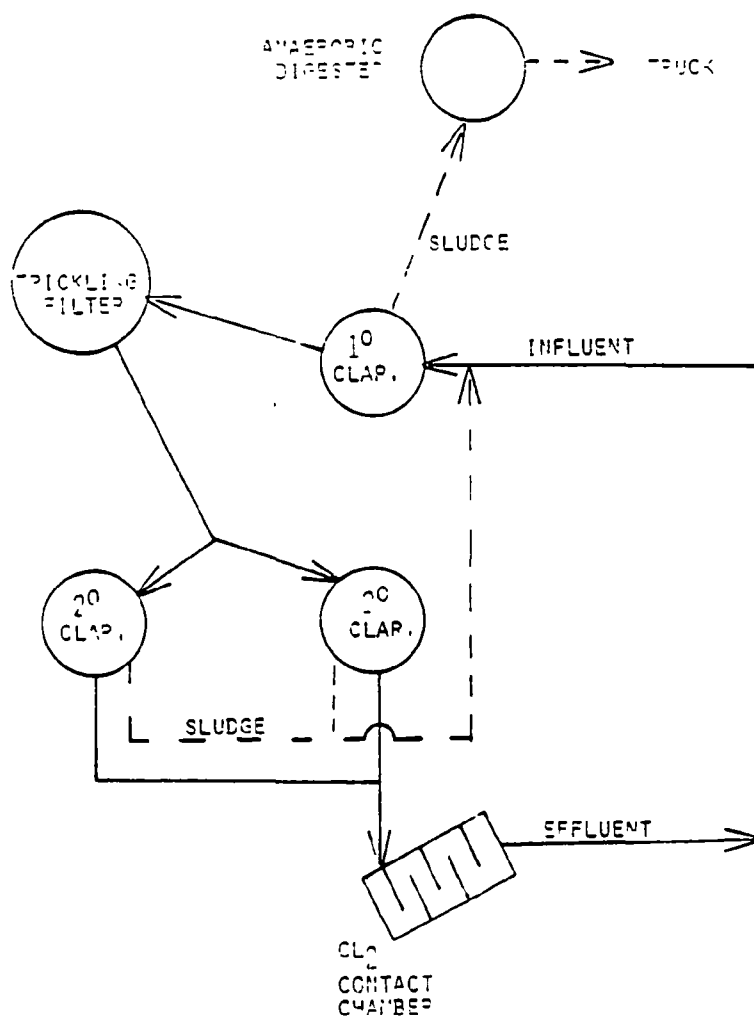


Figure B-14. Sewage Treatment Plant #19.

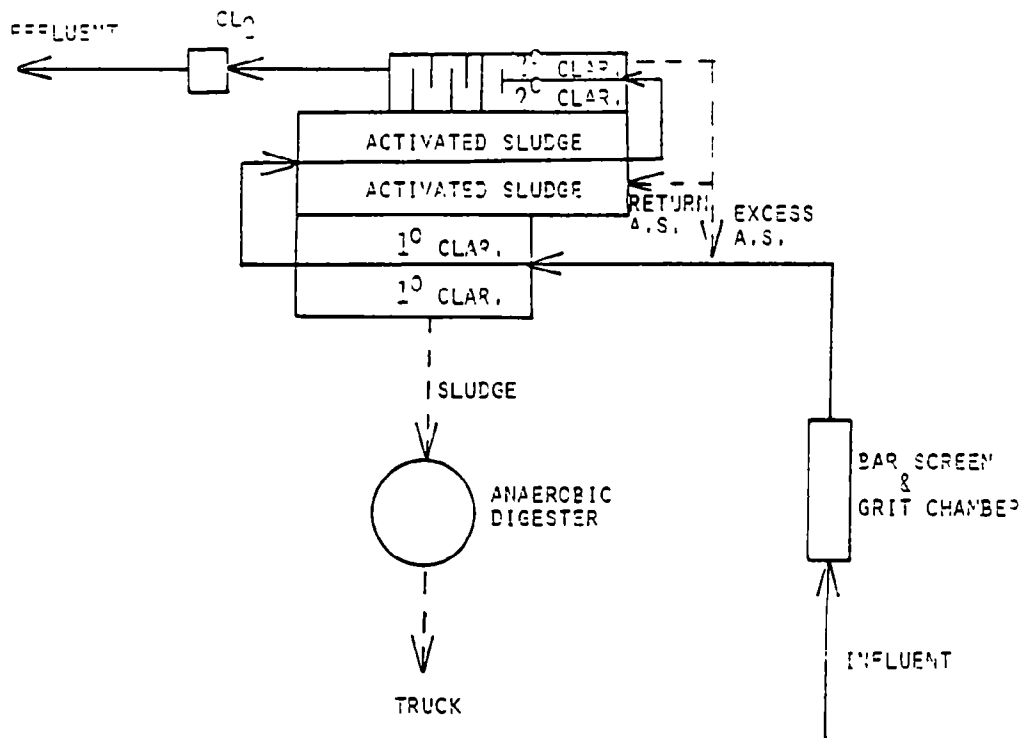


Figure 3-15. Sewage Treatment Plant '21.

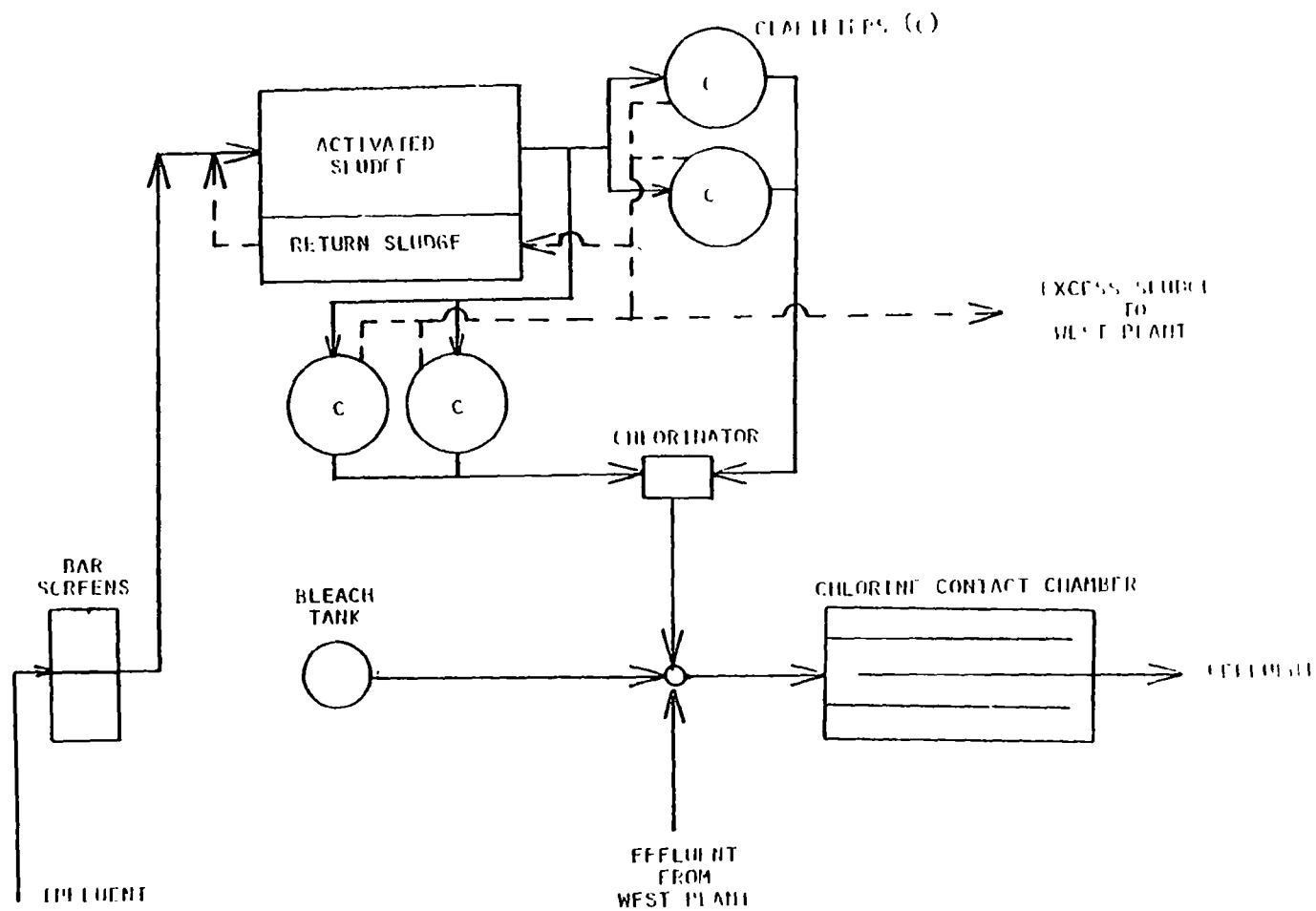


Figure B-16. Sewage Treatment Plant #22, East Plant.

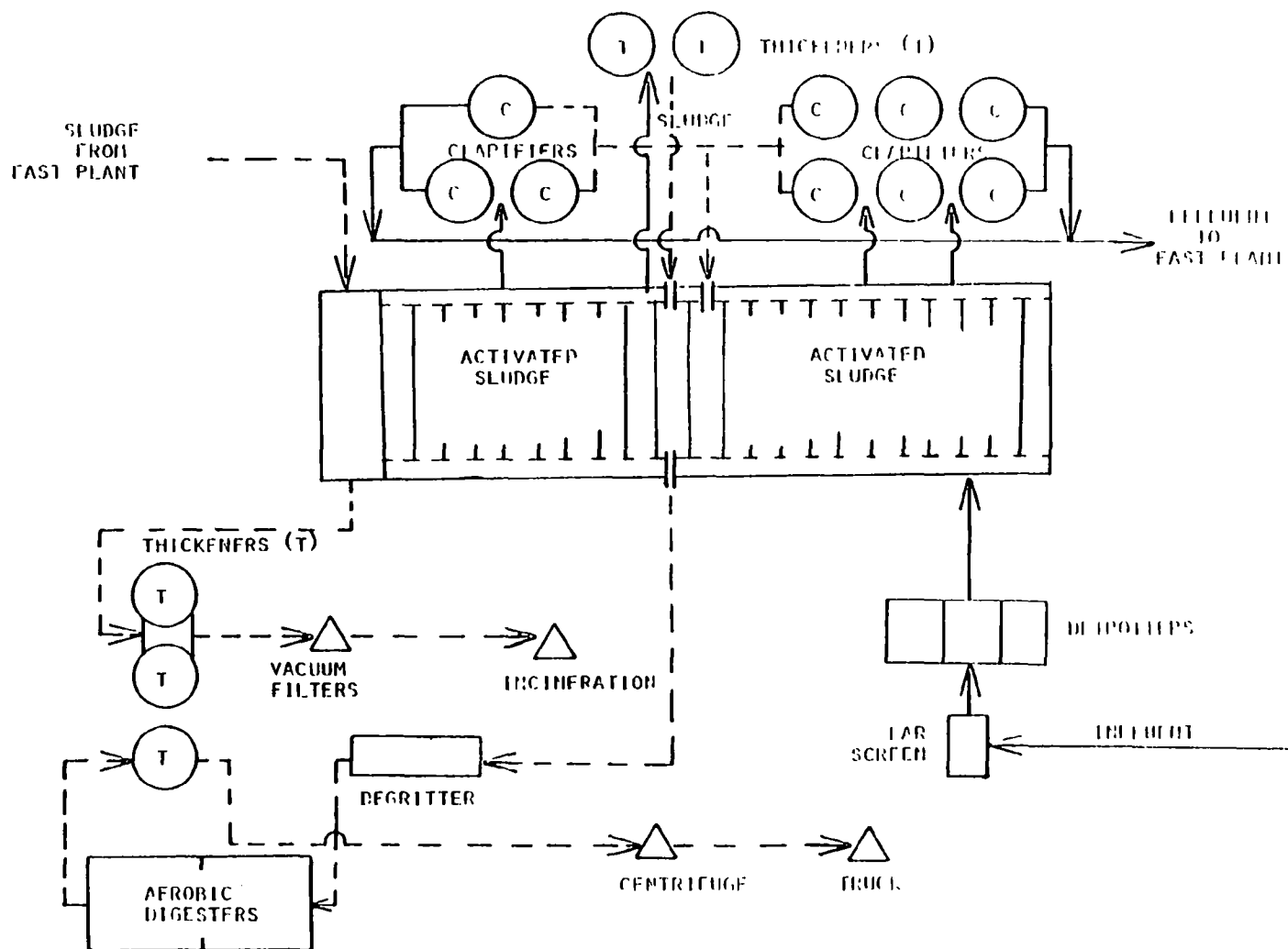


Figure B-17. Sewage Treatment Plant #22, West Plant.

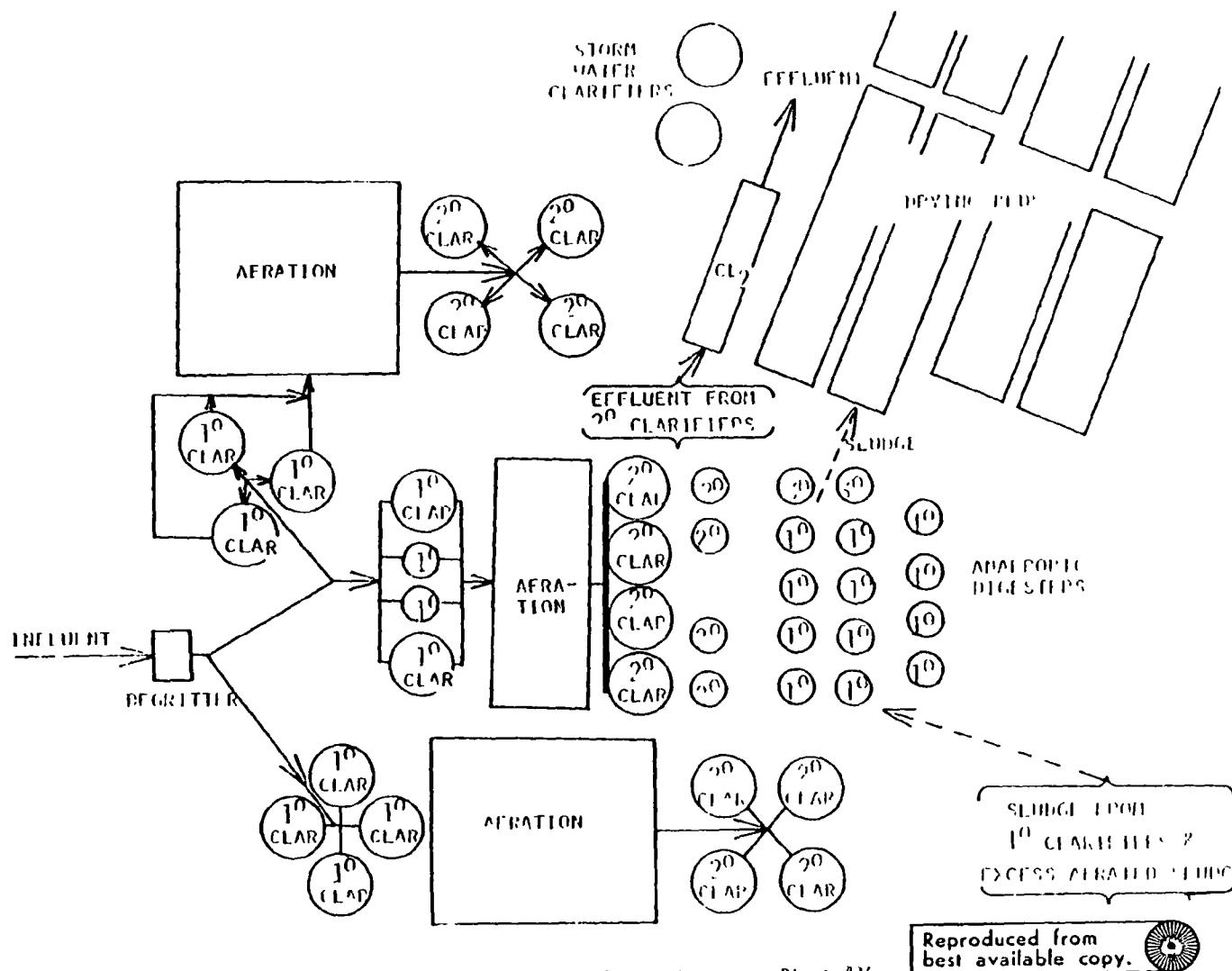


Figure B-18. Sewage Treatment Plant #24

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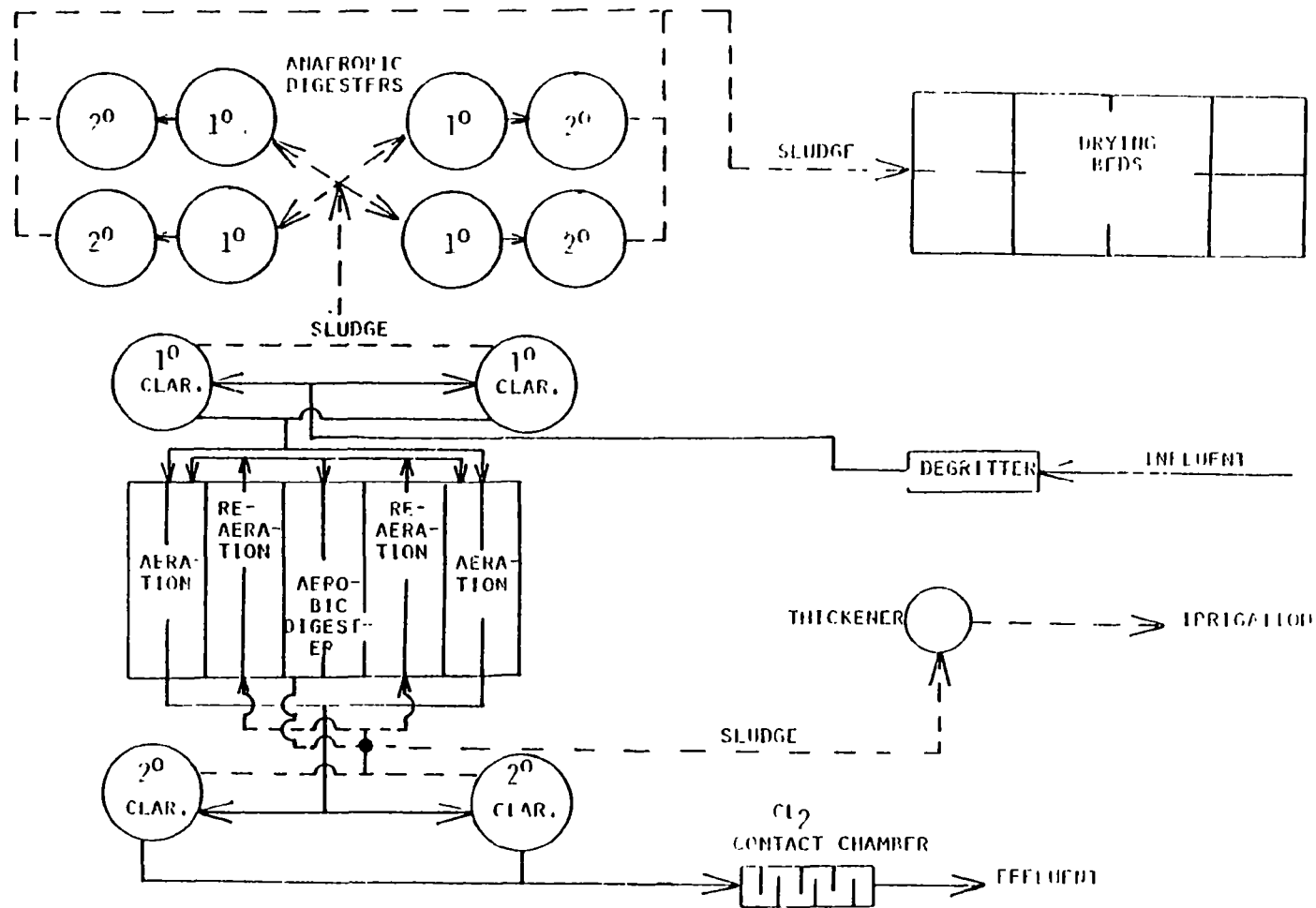


Figure B-19 Sewage Treatment Plant #25.

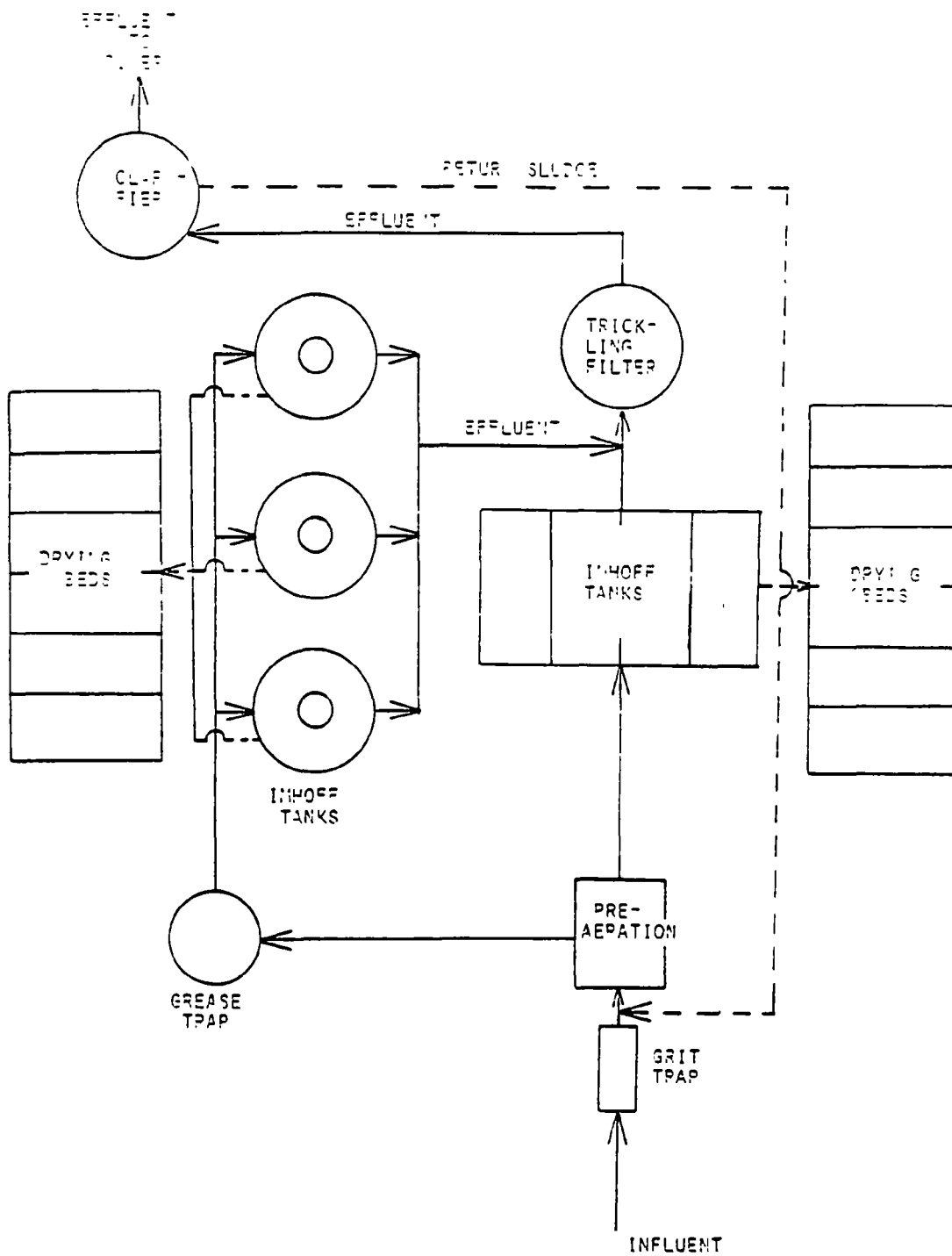


Figure 3-20. Sewage Treatment Plant #26.

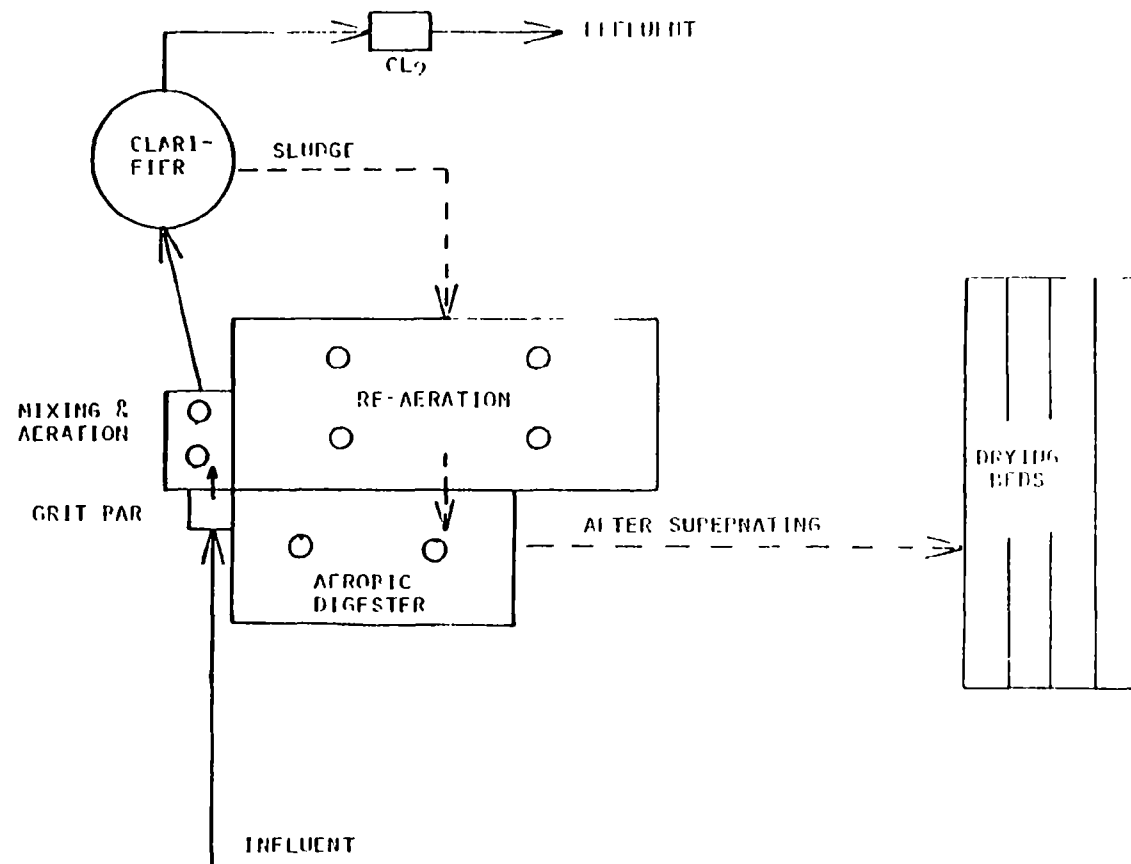


Figure B-21. Sewage Treatment Plant #8

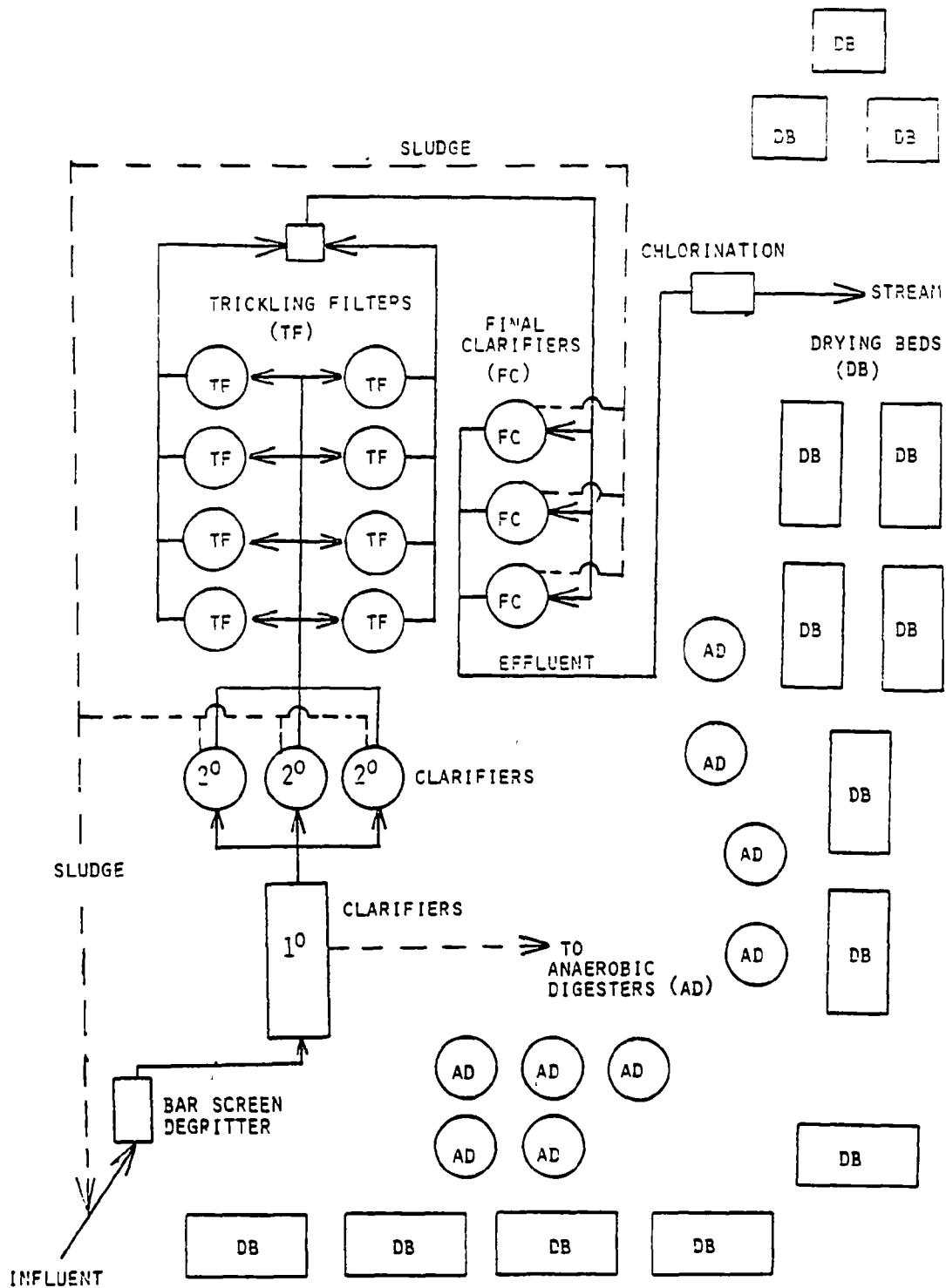


Figure B-22. Sewage Treatment Plant #30.

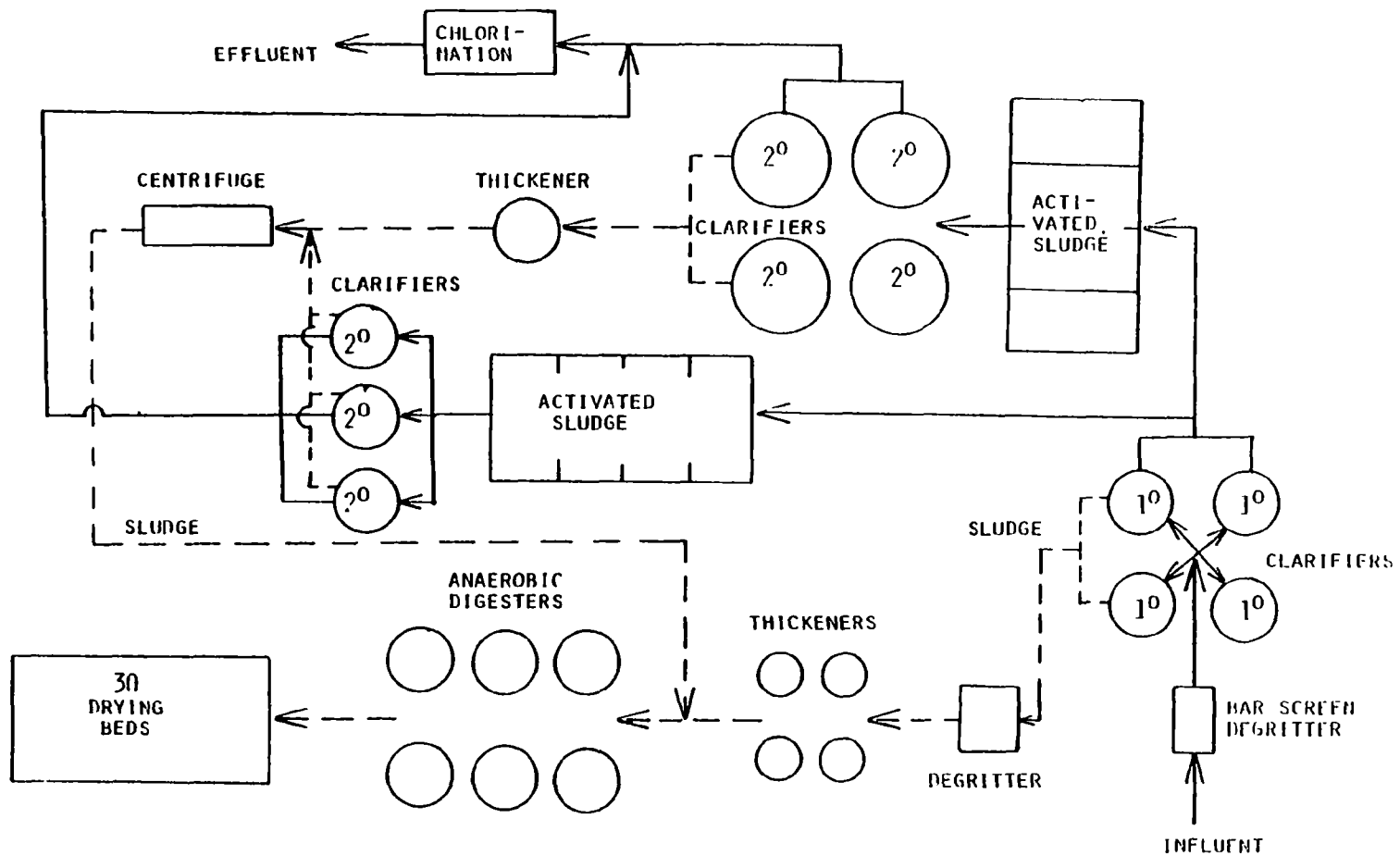


Figure B-23. Sewage Treatment Plant #31.

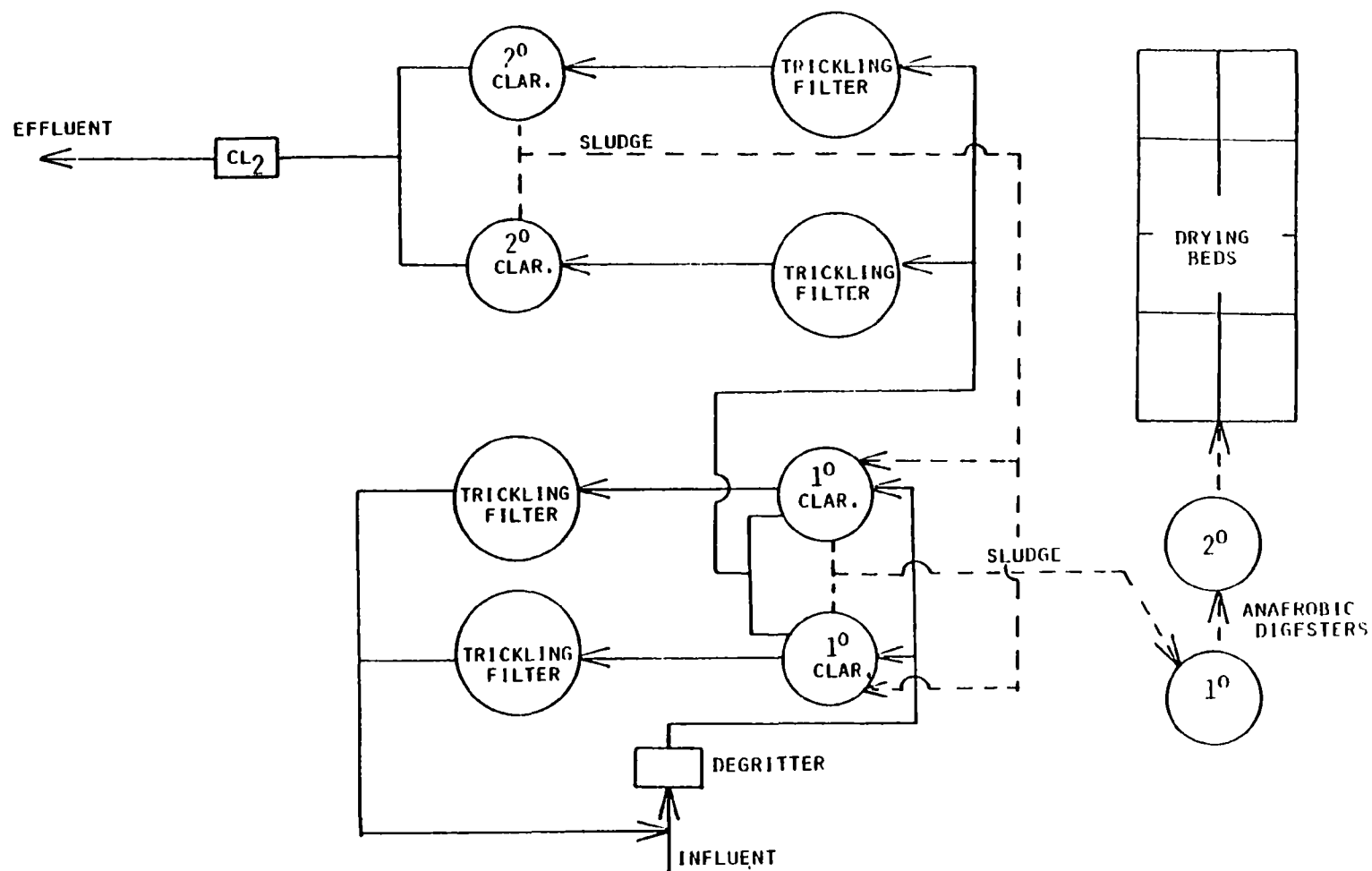


Figure B-24. Sewage Treatment Plant #34.

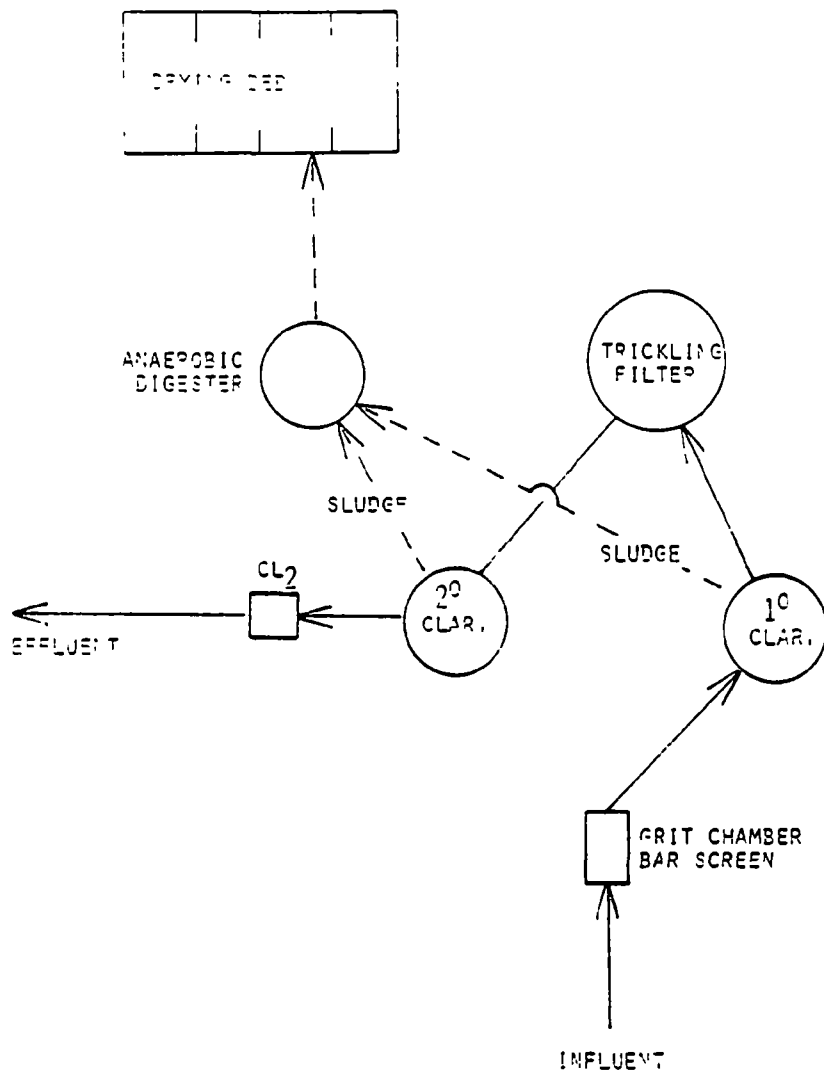


Figure 3-25. Sewage Treatment Plant #37.

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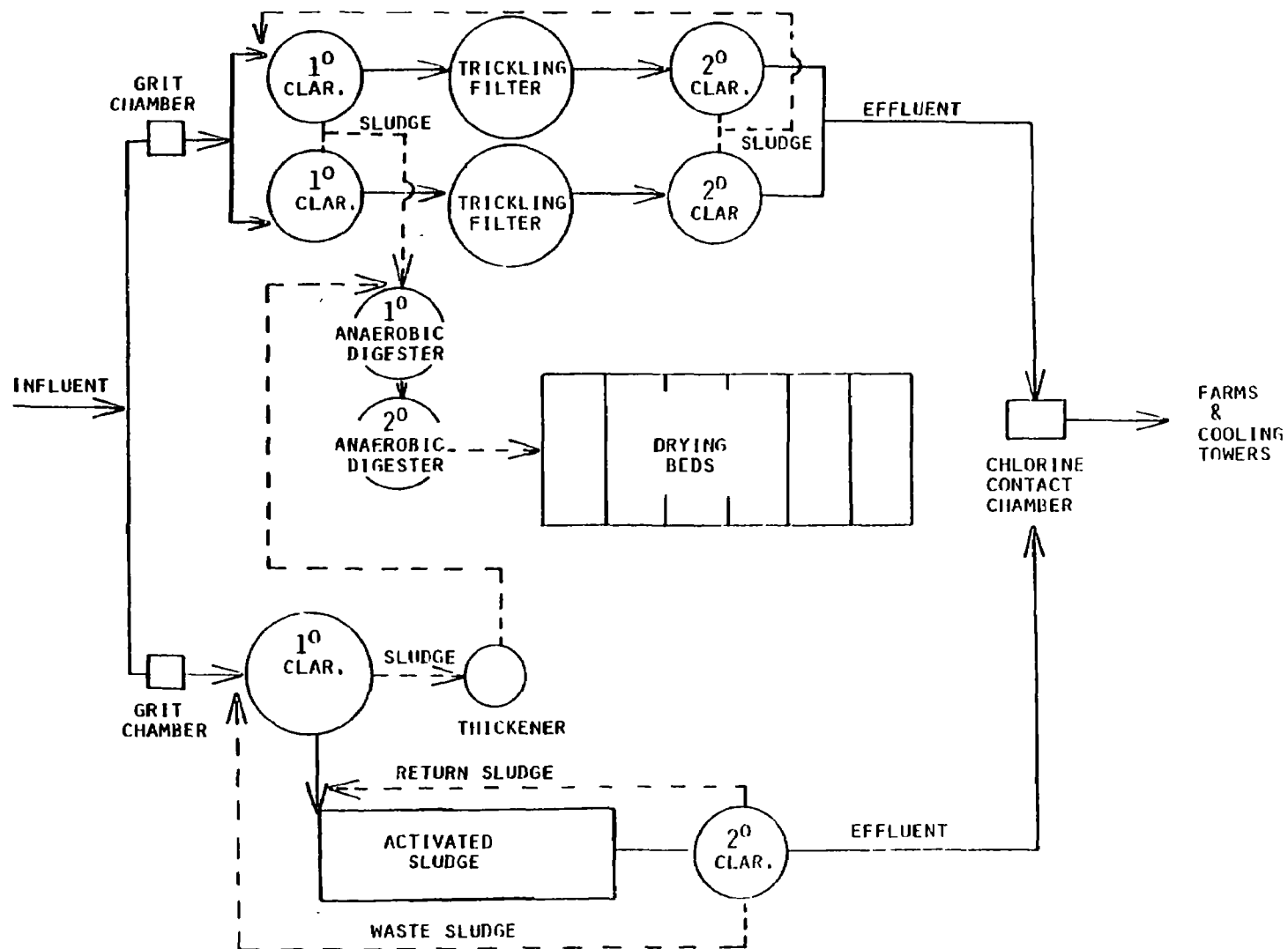


Figure B-26 Sewage Treatment Plant #38

APPENDIX C
RESULTS OF THE DRYING BED STUDY

TABLE C-1. HELMINTH EGGS (NO EGGS/KG DRY WT) FOUND IN SAMPLES FROM DIFFERENT LOCATIONS IN ONE DRYING BED (SEE DIAGRAM FOR LOCATIONS).

Sample	Percent Moisture Content	Actual Size Sample Examined gm dry wt.	Percent Solids	<u>Ascaris</u>		<u>T. trichiura</u>		<u>T. vulpis</u>		<u>Toxocara</u>	
				v	nv	v	nv	v	nv	v	nv
				(Total)		(Total)		(Total)		(Total)	
1	73.1	8.07	26.9	2,600	620	620	120	1,240	120	740	990
				(3220)		(740)		(1360)		(1730)	
2	25.9	22.23	74.1	1,260	180	130	40	850	130	180	40
				(1440)		(170)		(980)		(220)	
3	76.6	7.02	23.4	4,990	1,420	710	280	3,130	570	140	3,280
				(6410)		(990)		(3600)		(3420)	
4	72.0	8.40	28.0	3,930	480	480	0	1,550	240	360	1,900
				(8810)		(480)		(1790)		(2260)	
5	67.3	9.81	32.7	1,830	1,120	610	310	1,830	310	100	1,120
				(2950)		(920)		(2140)		(1220)	
6	74.2	7.74	25.8	2,970	390	390	130	1,810	0	260	1,420
				(3360)		(520)		(1810)		(1680)	
7	84.5	4.65	15.5	4,090	1,080	860	0	2,800	220	220	2,150
				(5170)		(860)		(3020)		(2370)	
8	73.5	7.95	26.5	4,530	1,380	380	250	3,140	630	130	3,270
				(5910)		(630)		(3770)		(3400)	
9	73.6	7.92	26.4	3,030	630	130	130	2,020	380	130	2,020
				(3660)		(260)		(2400)		(2150)	
10	80.8	5.76	19.2	4,340	1,390	170	170	1,390	170	520	1,560
				(5730)		(340)		(1560)		(2080)	

v = viable
nv = non-viable

TABLE C-2. HELMINTH EGGS (NO. EGGS/30 GM SAMPLE, WET WEIGHT) FOUND IN SAMPLES FROM DIFFERENT LOCATIONS IN ONE DRYING BLD (SEE DIAGRAM FOR LOCATION)

Sample	Actual Size Sample Examined gm dry wt.	<u>Ascaris</u>			<u>T. trichiura</u>			<u>L. vulpina</u>			<u>Toxocara</u>		
		v	nv	T	v	nv	T	v	nv	T	v	nv	T
1	8.07	21	5	26	5	1	6	10	1	11	6	8	14
2	22.23	28	1	29	3	1	4	19	3	22	4	9	13
3	7.02	35	10	45	5	2	7	22	4	26	1	23	24
4	8.40	33	4	37	4	0	4	13	2	15	3	16	19
5	9.81	18	11	29	6	3	9	18	3	21	1	11	12
6	7.74	23	3	26	3	1	4	14	0	14	2	11	13
7	4.65	19	5	24	4	0	4	11	1	14	1	10	11
8	7.95	36	11	47	3	2	5	25	5	30	1	26	27
9	7.92	24	5	29	1	1	2	16	3	19	1	16	17
10	5.76	25	8	33	1	1	2	8	1	10	3	9	12

v=viable
nv=non-viable
T=Total

TABLE C-3. STATISTICAL ANALYSIS OF DRYING BED SAMPLES
(NO EGGS/ΔG DRY WT) OF SLUDGE

Parameter	Median	Average	Standard Deviation	Range
Moisture (%) Content	73.0	71.0	5.0	34.5-67.5
<u>Ascaris</u>				
Total	5170	4226	1572	6710-2950
Viable	3930	3590	1034	4986-1835
non-viable	1080	946	417	1420-390
<u>T. trichiura</u>				
Total	630	638	258	990-260
Viable	480	483	242	860-130
Non-viable	130	154	111	310-0
<u>T. vulpis</u>				
Total	2140	2383	886	3770-1360
Viable	1830	2101	736	3140-1240
Non-viable	240	293	205	630-0
<u>Toxocara</u>				
Total	2150	2257	742	3420-1220
Viable	220	289	217	740-100
Non-viable	1900	1968	837	3280-990

APPENDIX D

CALCULATIONS ON THE EFFECTIVENESS OF WASTEWATER TREATMENT PROCESS
ON PARASITE REDUCTIONS IN THE FIELD

TABLE D-1 PERCENT REDUCTION OF VIABLE ASCARIS EGGS IN FIELD SAMPLES
COLLECTED FROM WASTEWATER TREATMENT PLANTS

Plant	Fall	Winter	Spring	Summer	Sludge Treatment
1	85	53	52	46	Anaerobic
2	-	100	69	10	Anaerobic
3	-	-	-	-	Aerobic
4	-	(296)	100	100	Anaerobic
6	100	86	100	100	Aerobic
7	-	8	26	59	Aerobic
8	100	100	100	44	Anaerobic
10	(142)	(9)	100	82	Anaerobic
11	99	89	100	86	Anaerobic
13	-	-	100	-	Aerobic
14	-	-	-	100	Anaerobic
16	63	93	100	100	Anaerobic
17	-	-	-	-	Aerobic
18	(250)	93	100	97	Anaerobic
19	-	-	-	-	Anaerobic
21	100	100	100	-	Anaerobic
24	93	94	22	100	Anaerobic
25	-	-	-	100	Both
26	93	95	98	100	Anaerobic
28	100	80	(-)	-	Aerobic
29	91	(40)	100	-	Aerobic
30	-	-	-	-	Anaerobic
31	100	-	100	100	Anaerobic
34	100	99	100	89	Anaerobic
37	93	(138)	99	100	Anaerobic
38	100	-	-	100	Anaerobic
		<u>TOTAL</u>			<u>ALL SEASONS</u>
N	16	17	18	18	69
Average	58	36	87	84	67
Standard Deviation	101	108	26	26	76
		<u>Aerobic</u>			
N	3	4	4	2	13
Average	97	34	82	80	70
Standard Deviation	5	60	37	29	45
		<u>Anaerobic</u>			
N	13	13	14	15	55
Average	49	37	89	84	66
Standard Deviation	111	121	24	28	83

TABLE D-2. PERCENT REDUCTION OF VIABLE TOXOCARA EGGS

Plant	Fall	Winter	Spring	Summer	Sludge Treatment
1	100	100	92	100	Anaerobic
2	-	100	100	94	Anaerobic
3	-	-	-	-	Aerobic
4	-	100	100	-	Anaerobic
6	-	100	100	100	Aerobic
7	33	-	43	-	Aerobic
8	97	100	96	75	Anaerobic
10	(41)	77	-	100	Anaerobic
11	100	100	100	-	Anaerobic
13	-	100	100	100	Aerobic
14	-	97	100	100	Anaerobic
16	-	94	100	-	Anaerobic
17	-	-	-	-	Aerobic
18	43	97	100	100	Anaerobic
19	-	-	-	-	Anaerobic
21	-	100	-	-	Anaerobic
24	100	100	80	100	Anaerobic
25	100	100	-	100	Both
26	100	88	100	-	Anaerobic
28	100	97	95	100	Aerobic
29	74	94	100	100	Aerobic
30	100	97	100	100	Anaerobic
31	100	93	100	100	Anaerobic
34	100	97	100	100	Anaerobic
37	100	(76)	100	100	Anaerobic
38	100	100	-	100	Anaerobic
		<u>TOTAL</u>			<u>ALL SEASONS</u>
N	16	22	19	17	74
Average	82	89	95	98	91
Standard Deviation	39	37	14	6	28
		<u>Aerobic</u>			
N	3	4	5	4	16
Average	69	98	88	100	90
Standard Deviation	34	3	25	0	21
		<u>Anaerobic</u>			
N	12	17	14	12	55
Average	83	86	98	97	91
Standard Deviation	42	42	6	7	31

TABLE D-3. PERCENT REDUCTION OF TABLE *TRICHLURIS TRICHLURA* EGGS

Plant	Fall	Winter	Spring	Summer	Sludge Treatment
1	100	100	91	-	Anaerobic
2	-	-	-	-	Anaerobic
3	-	-	-	-	Aerobic
4	-	-	-	-	Anaerobic
6	-	-	-	-	Aerobic
7	(66)	(155)	54	67	Aerobic
8	-	-	-	-	Anaerobic
10	-	-	-	-	Anaerobic
11	-	-	-	100	Anaerobic
13	-	-	-	-	Aerobic
14	-	-	-	-	Anaerobic
16	-	-	-	-	Anaerobic
17	-	-	-	-	Aerobic
18	(95)	96	100	100	Anaerobic
19	(13)	(10)	37	-	Anaerobic
21	100	100	-	-	Anaerobic
24	-	-	-	100	Anaerobic
25	-	-	-	-	Both
26	-	-	-	-	Anaerobic
28	-	-	-	-	Aerobic
29	-	-	-	-	Aerobic
30	-	-	-	-	Anaerobic
31	-	-	-	-	Anaerobic
34	100	100	100	-	Anaerobic
37	93	(416)	99	100	Anaerobic
38	-	-	-	-	Anaerobic
	<u>TOTAL</u>				<u>ALL SEASONS</u>
N	7	7	6	5	26
Average	31	(26)	80	93	39
Standard Deviation	87	196	28	15	118
	<u>Aerobic</u>				
N	1	1	1	1	4
Average	(66)	(155)	54	67	(25)
Standard Deviation	-	-	-	-	105
	<u>Anaerobic</u>				
N	6	6	5	4	21
Average	48	(5)	85	100	52
Standard Deviation	82	206	27	0	119

TABLE D-4 PERCENT REDUCTION OF VIABLE TRICHURI MULPIS EGGS

Plant	Fall	Winter	Spring	Summer	Sludge Treatment
1	-	(122)	20	42	Anaerobic
2	-	96	35	100	Anaerobic
3	-	-	-	-	Aerobic
4	-	(273)	98	100	Anaerobic
6	-	-	-	100	Aerobic
7	-	-	57	(13)	Aerobic
8	87	100	91	-	Anaerobic
10	(255)	(16)	100	55	Anaerobic
11	-	(27)	87	100	Anaerobic
13	-	100	100	-	Aerobic
14	-	-	(41)	100	Anaerobic
16	-	(136)	-	-	Anaerobic
17	-	-	-	-	Aerobic
18	(167)	10	91	82	Anaerobic
19	-	-	-	-	Anaerobic
21	-	100	100	-	Anaerobic
24	-	-	-	-	Anaerobic
25	100	100	87	100	Both
26	94	77	93	100	Anaerobic
28	-	-	3	83	Aerobic
29	81	(5)	92	100	Aerobic
30	-	-	100	100	Anaerobic
31	-	-	-	-	Anaerobic
34	9	(27)	89	-	Anaerobic
37	44	43	63	(68)	Anaerobic
38	-	100	-	100	Anaerobic
		<u>TOTAL</u>			<u>ALL SEASONS</u>
N	8	16	18	16	58
Average	(1)	7	70	74	44
Standard Deviation	135	108	41	49	87
		<u>Aerobic</u>			
N	1	2	4	4	11
Average	81	48	63	68	63
Standard Deviation	-	74	44	54	46
		<u>Anaerobic</u>			
N	6	13	13	11	43
Average	(31)	(7)	71	74	34
Standard Deviation	145	113	42	51	96

TABLE D-5 PERCENT REDUCTION OF VIABLE ASCARIS EGGS BY DIGESTION
PROCESS IN FIELD SAMPLES FOR UNIT OPERATIONS

Plant	Fall	Winter	Spring	Summer	Process
13	-	-	-	9	Aerobic
17	-	23	13	(307)	Thickeners
19	(150)	(8)	(116)	(313)	Anaerobic
21	-	-	(88)	(33)	Anaerobic
	-	-	100	-	Drying Bed
22	(350)	(33)	(155)	(148)	Aerobic
	-	92	44	47	Vacuum Filtration
	-	87	-	(13)	Centrifugation
25	-	-	-	(48)	Aerobic
	100	-	100	100	Drying Bed
26	-	-	-	-	Anaerobic
	93	95	98	100	Drying Bed
29	-	-	-	-	Aerobic
	91	(40)	100	-	Drying Bed

	<u>Aerobic Digestion</u>	<u>Anaerobic Digestion</u>
N	6	6
Average	(121)	(118)
Standard Deviation	130	109

	<u>Drying Beds</u>
N	6
Average	75
Standard Deviation	57

	<u>Dewatering (All Aerobic)</u>		
	<u>Thickening</u>	<u>Vacuum Filtration</u>	<u>Centrifugation</u>
N	3	3	2
Average	(110)	61	37
Standard Deviation	222	27	71

TABLE D-6 PERCENT REDUCTION OF TABLE TOXOCARA EGGS BY
DIGESTION PROCESS IN FIELD SAMPLES FOR UNIT OPERATIONS

Plant	Fall	Winter	Spring	Summer	Process
13	41	(113)	-	100	Aerobic
	-	100	-	-	Drying Bed
17	-	(12)	67	(38)	Thickeners
19	59	(233)	56	-	Anaerobic
21	-	-	-	-	Anaerobic
	-	-	100	-	Drying Bed
22	(70)	67	33	-	Aerobic
	-	100	(12)	(118)	Vacuum Filtration
	(24)	83	-	100	Centrifugation
25	42	45	-	(121)	Aerobic
	100	100	-	100	Drying Bed
26	-	-	-	-	Aerobic
	100	88	100	-	Drying Bed
29	-	-	-	-	Aerobic
	74	94	100	100	Drying Bed

	<u>Aerobic Digestion</u>	<u>Anaerobic Digestion</u>
N	9	3
Average	(167)	(36)
Standard Deviation	82	171

	<u>Drying Beds</u>	
N	8	4
Average	96	97
Standard Deviation	9	6

	<u>Dewatering (All Aerobic)</u>		
	<u>Thickening</u>	<u>Vacuum Filtration</u>	<u>Centrifugation</u>
N	3	3	3
Average	6	(16)	53
Standard Deviation	55	109	67

TABLE D-7. PERCENT REDUCTION OF VIABLE TRICHURIS TRICHIURA EGGS BY
DIGESTION PROCESS IN FIELD SAMPLES FOR UNIT OPERATIONS

Plant	Fall	Winter	Spring	Summer	Process
13	-	-	-	-	Aerobic
	-	-	-	-	Drying Beds
17	-	-	-	-	Thickeners
19	(13)	(10)	37	-	Anaerobic
21	-	-	-	(400)	Anaerobic
	-	-	100	-	Drying Bed
22	-	100	-	-	Aerobic
	-	-	-	100	Vacuum Filtration
	-	-	-	100	Centrifugation
25	-	-	-	-	Aerobic
	-	-	-	-	Drying Beds
26	-	-	-	-	Anaerobic
	-	-	-	-	Drying Beds
29	-	-	-	-	Aerobic
	-	-	-	-	Drying Beds

	<u>Aerobic Digestion</u>	<u>Anaerobic Digestion</u>
N	1	4
Average	100	(97)
Standard Deviation	-	204
	<u>Drying Beds</u>	
N	-	1
Average	-	100
Standard Deviation	-	-
	<u>Dewatering (All Aerobic)</u>	
	<u>Thickening</u>	<u>Vacuum Filtration</u> <u>Centrifugation</u>
N	-	1 1
Average	-	100 100
Standard Deviation	-	- -

TABLE 2-3 PERCENT REDUCTION OF TABLE TRICHOCEPHALUS EGG
IN FIELD SAMPLES FOR UNIT OPERATIONS

Plant	Fall	Winter	Spring	Summer	Process
13	-	(55)	-	(122)	Aerobic
	-	100	-	-	Drying Beds
17	-	-	-	-	Thickens
19	(157)	10	91	82	Anaerobic
21	-	-	46	-	Anaerobic
	-	-	100	-	Drying Beds
22	-	-	55	-	Aerobic
	-	100	(47)	78	Vacuum filtration
	0	100	-	23	Centrifugation
23	100	100	(100)	100	Aerobic
	-	-	93	-	Drying Beds
26	-	-	-	-	Anaerobic
	94	77	93	100	Drying Beds
29	-	-	-	-	Aerobic
	81	(5)	92	100	Drying Beds

	<u>Aerobic Digestion</u>	<u>Anaerobic Digestion</u>	
N	7	5	
Average	11	12	
Standard Deviation	100	105	
	<u>Drying Bed</u>		
N	6	5	
Average	77	93	
Standard Deviation	41	9	
	<u>Dewatering (All Aerobic)</u>		
	<u>Thickening</u>	<u>Vacuum Filtration</u>	<u>Centrifugation</u>
N	-	3	2
Average	-	44	62
Standard Deviation	-	79	34

*() = -Value

APPENDIX E

RESULTS OF LABORATORY STUDIES CONTAINING RAW AND ANALYZED DATA

TABLE E-1 EFFECTS OF AFROBIC DIGESTION ON ASCARIS EGGS (FROM THE UTERI OF WORMS)
AND TOXOCARA EGGS (FROM DOG FECES)

Time (Days)	Ascaris Eggs/100ml ¹		Percent ³ Reduction Viable Eggs	Percent ⁴ Recovery of Total Eggs		Toxocara Eggs/100ml ¹		Percent ³ Reduction Viable Eggs	Percent ⁴ Recovery of Total Eggs
	V	NV		% Viable ²	% Viable ²	V	NV		
	Digester at 35°C (Run #1)								
0.04 ⁵	103	48	-	68	38 (400)	134	98	-	67 58 (400)
2	0	1	100	0	0 (324)	2	68	98	1 22 (324)
4	0	0	100	0	0 (262)	10	4	87	71 5 (252)
6	0	0	100	0	0 (213)	8	4	89	67 6 (213)
8	0	0	100	0	0 (172)	0	3	100	0 2 (172)
10	0	0	100	0	0 (140)	7	0	85	0 5 (140)
Digester at 35°C (Run #2)									
0.04 ⁵	207	26	-	89	58 (400)	186	153	-	55 85 (400)
2	0	2	100	0	1 (324)	15	21	77	63 17 (324)
4	0	0	100	0	0 (262)	10	14	92	42 9 (262)
6	0	0	100	0	0 (213)	2	0	99	100 1 (213)
8	0	0	100	0	0 (172)	1	1	99	50 1 (172)
10	1	0	99	100	1 (140)	3	0	95	100 2 (140)
Digester at 45°C (Run #1)									
0.04 ⁵	190	3	-	98	39 (500)	205	206	-	50 82 (500)
2	6	8	97	43	4 (405)	39	18	76	68 14 (405)
4	1	6	99	14	2 (328)	41	24	70	63 20 (328)
6	1	0	99	100	0 (266)	31	23	72	57 20 (266)
8	3	1	96	100	2 (215)	17	9	81	65 12 (215)
10	0	1	100	0	0 (174)	19	4	73	83 13 (174)

(continued)

TABLE F-1. (continued)

TABLE 1. (Continued)										
Time (Days)	Ascaris Eggs/100ml ¹		Percent ³ Reduction Viable Eggs	Z Viable ²	Percent ⁴ Recovery of Total Eggs	Toxocara Eggs/100ml ¹		Percent ³ Reduction Viable Eggs	Viable	Percent ⁴ Recovery of Total Eggs
	V	NV				V	NV			
Digester at 45°C (Run #2)										
0.04 ⁵	119	11	-	92	26 (500)	185	195	-	49	76 (500)
2	2	2	98	50	1 (405)	86	40	43	68	61 (405)
4	1	2	99	33	1 (328)	18	26	69	59	20 (328)
6	0	0	100	0	0 (266)	31	15	69	67	17 (266)
8	0	0	100	0	0 (215)	21	23	74	48	20 (215)
10	0	0	100	0	0 (174)	28	11	57	77	22 (174)
Digester at 55°C (Run #1)										
0.04 ⁵	0	273	-	0	55 (500)	0	435	-	0	87 (500)
2	0	145	100	0	36 (405)	0	317	100	0	77 (405)
4	0	128	100	0	19 (328)	0	202	100	0	62 (328)
6	0	56	100	0	21 (266)	0	158	100	0	59 (266)
8	0	88	100	0	41 (215)	0	131	100	0	61 (215)
10	0	79	100	0	45 (174)	0	63	100	0	16 (174)
Digester at 55°C (Run #2)										
0.04 ⁵	0	175	-	0	35 (500)	0	351	-	0	70 (500)
2	0	193	100	0	48 (405)	0	339	100	0	84 (405)
4	0	165	100	0	50 (326)	0	240	100	0	73 (326)
6	0	127	100	0	48 (266)	0	203	100	0	76 (266)
8	0	94	100	0	44 (215)	0	146	100	0	68 (215)
10	0	66	100	0	38 (174)	0	51	100	0	29 (174)

¹V = Viable eggs; NV = Non-Viable eggs²Z Viable = Percent viable eggs found in the sample as compared to total number of eggs observed³Percent Reduction = Z viable eggs noted to be reduced from the number of viable eggs found at 1 hr of operation⁴Percent Recovery = Z of the total number of eggs recovered to the total theoretical number of eggs (illustrated in parentheses) that should be found, assuming decrease is due only to dilution⁵These samples were neutralized, then refrigerated for 5 to 12 days before parasite analysis

TABLE E-2. (continued)

Time (Days)	Ascaris Eggs/100ml ¹		Percent ³ Reduction Viable Eggs	Percent ⁴ Recovery of Total Eggs	Percent ⁴ Recovery of Total Eggs	Ascaris Eggs/100ml ¹		Percent ³ Reduction Viable Eggs	Percent Viable
	V	NV				V	NV		
<u>Digester at 45°C</u>									
0.04	774	40	-	95	82 (990)	2	0	-	100
2 ⁵	527	13	16	98	67 (802)	0	0	-	-
	[634] ⁶	[102] ⁶	[0] ⁶	[86] ⁶	[92 (802)] ⁶				
4 ⁵	0	602	100	0	93 (650)	0	0	-	-
6 ⁵	0	468	100	0	89 (526)	0	2	-	0
8	0	116	100	0	27 (426)	0	0	-	-
	[0] ⁶	[238] ⁶	[100] ⁶	[0] ⁶	[55 (426)] ⁶				
10	0	358	100	0	104 (345)	0	0	-	-
<u>Digester at 55°C</u>									
0.04	706	230	-	75	95 (990)	0	18	-	0
2 ⁵	0	826	100	0	103 (802)	0	16	-	0
4 ⁵	0	630	100	0	97 (650)	0	6	-	0
6 ⁵	0	320	100	0	61 (526)	0	12	-	0
	[0] ⁶	[550] ⁶	[100] ⁶	[0] ⁶	[129 (526)] ⁶				
8	0	350	100	0	82 (426)	0	0	-	-
10	0	326	100	0	94 (345)	0	6	-	0

¹V = Viable eggs; NV = Non-Viable eggs.²Percent Viable = Percent viable eggs found in sample as compared to total number of eggs observed.³Percent Reduction = Percent viable eggs noted to be reduced from number of viable eggs found at 1 hr of operation.⁴Percent Recovery = Percent of total number of eggs recovered to the total theoretical number of eggs (illustrated in parentheses) that should be found assuming decrease is due only to dilution.⁵These samples were neutralized, then refrigerated 5 to 12 days before parasite analysis.⁶[Second sample analyzed again a week after the first sample.]

TABLE E-2. EFFECTS OF AEROBIC DIGESTION ON ASCARIS EGGS
(FROM THE SMALL INTESTINAL CONTENTS OF SWINE)

Time (Days)	Ascaris Eggs/100ml ¹		Percent ³ Reduction Viable Eggs	Percent ² Viable	Percent ⁴ Recovery of Total Eggs	Ascaris Eggs/100ml ¹		Percent ³ Reduction Viable Eggs	Percent ⁴ Viable
	V	NV				V	NV		
<u>Digester at 28°C</u>									
0.04	758	22	-	97	97 (990)	4	8	-	33
2 ⁵	436	20	29	96	57 (802)	26	4	-	87
	[930] ⁶	[104] ⁶	[0] ⁶	[90] ⁶	[129 (802)] ⁶				
4 ⁵	572	28	0	95	92 (650)	10	0	-	100
6 ⁵	372	2	0	99	71 (526)	0	0	-	-
8	424	6	0	99	101 (426)	0	0	-	-
10	324	0	0	100	94 (345)	2	0	-	100
<u>Digester at 35°C</u>									
0.04	744	2	-	100	75 (990)	0	0	-	-
	[992] ⁶	[104] ⁶	[0] ⁶	[91] ⁶	[111 (990)] ⁶				
2 ⁵	866	6	0	99	109 (802)	0	0	-	-
4 ⁵	668	2	0	100	103 (650)	8	4	-	67
6 ⁵	378	6	0	98	73 (526)	0	0	-	-
	[436] ⁶	[26] ⁶	[0] ⁶	[94] ⁶	[88 (526)] ⁶				
8	490	2	0	100	115 (426)	0	0	-	-
10	276	12	10	96	83 (345)	0	0	-	-

(continued)

TABLE F-3. EFFECTS OF ANAEROBIC DIGESTION ON ASCARIS EGGS (FROM THE SMALL INTESTINAL CONTENTS OF SWINE) AND TOXOKARA EGGS (FROM DOG FECES) IN RAW SECONDARY SLUDGE

Ascaris							Toxocara					
Time (days)	Expected Eggs/50 ml	Eggs/50 ml		% Decrease v. Eggs ²	% v. Eggs ³	% Recovery ^b	Expected Eggs/50 ml	Eggs/50 ml		% Decrease v. Eggs ²	% v. Eggs ³	% Recovery ^b
		V	NV ¹					V	NV ¹			
TEST - DIGESTER 35°C												
0.010	197	112	46	-	71	80	181	140	12	-	92	84
2	167	90	19	5.2	83	65	154	143	17	0	89	103
6	121	48	15	30	76	52	111	70	14	18	83	76
10	87	43	12	13	78	63	80	44	7	29	86	81
15	63	21	8	42	72	46	58	25	10	44	71	60
CONTROL - DIGESTER 35°C												
0.010	-	11	1	-	92	-	-	10	0	-	100	-
2	-	2	1	-	67	-	-	8	1	-	89	-
6	-	6	1	-	86	-	-	-	0	-	100	-
10	-	5	0	-	100	-	-	1	0	-	100	-
15	-	1	1	-	50	-	-	0	0	-	-	-
TEST - DIGESTER 45°C												
0.014	197	80	43	-	65	62	181	129	64	-	67	106
2	167	0	92	100	0	55	154	0	102	100	0	66
6	121	0	89	100	0	74	111	0	67	100	0	60
10	87	0	45	100	0	52	80	0	41	100	0	51
15	63	0	24	100	0	38	58	0	30	100	0	-

(continued)

TABLE E-3 (continued)

Ascaris													Toxocara				
Time (days)	Expected Eggs/50 ml	Eggs/50 ml		% Decrease V. Eggs ²	% V. Eggs ³	% Recovery ⁴	Expected Eggs/50 ml	Eggs/50 ml		% Decrease V. Eggs ²	% V. Eggs ³	% Recovery ⁴					
		V	NV ¹					V	NV ¹								
CONTROL - DIGESTER 45°C																	
0.014	-	5	3	-	63	-	-	8	1	-	89	-					
2	-	1	2	-	33	-	-	3	2	-	60	-					
6	-	2	0	-	100	-	-	3	0	-	100	-					
10	-	0	0	-	-	-	-	1	1	-	50	-					
15	-	0	1	-	-	-	-	0	0	-	-	-					
TEST - DIGESTER 55°C																	
0.017	193	0	92	-	0	48	194	1	147	-	0	76					
2	164	0	94	100	0	57	165	0	80	100	0	48					
6	119	0	47	100	0	19	119	0	35	100	0	29					
10	86	0	37	100	0	43	86	0	4	100	0	47					
15	62	0	11	100	0	18	62	0	2	100	0	32					
CONTROL - DIGESTER 55°C																	
0.017	-	4	6	-	40	-	-	2	6	-	25	-					
2	-	0	2	-	0	-	-	1	0	-	100	-					
6	-	0	2	-	0	-	-	0	0	-	-	-					
10	-	0	2	-	0	-	-	0	0	-	-	-					
15	-	0	0	-	-	-	-	0	0	-	-	-					

¹V = Viable, NV = Non-Viable.²Percent noted to be reduced from number found in first sample³As compared to total number of eggs observed⁴Percent recovered to the total expected number, assuming decrease is due only to dilution

TABLE E-4. EFFECTS OF LIME STABILIZATION ON ASCARIS EGGS
(FROM THE SMALL INTESTINAL CONTENTS) IN PRIMARY
SLUDGE UNDER AEROBIC CONDITIONS AT
AMBIENT TEMPERATURE.

Time (Days)	Ascaris Eggs/50ml ¹		% Red ³ Viable Eggs	% Viable ²	% Rec. ⁴ Total Eggs	pH
	V	NV				
<u>No Lime Dosage</u>						
0.04	48	0	-	100	73(66)	6.1
5 ⁵	51	2	0	96	80(66)	6.7
10	15	3	69	83	27(66)	7.3
15 ⁵	10	3	79	77	20(66)	6.8
20 ⁵	2	0	96	100	3(66)	8.6
<u>100 mg of Lime (Ca(OH)₂) Per Gram Susp. Solids</u>						
0.04	37	0	-	100	56(66)	12.5
5 ⁵	33	4	11	89	56(66)	7.9
10	18	1	51	95	29(66)	8.5
15	-	-	-	-	-	-
20	-	-	-	-	-	-
<u>1000 mg Lime (Ca(OH)₂) Per Gram Susp. Solids</u>						
0.04	45	7	-	87	79(66)	13.2
5 ⁵	9	24	80	27	50(66)	13.1
10	0	4	100	0	6(66)	13.0
15 ⁵	0	0	100	0	0(66)	13.0
20 ⁵	0	1	100	0	2(66)	11.4
<u>3000 mg Lime (Ca(OH)₂) Per Gram Susp. Solids</u>						
0.04	51	8	-	98	79(66)	13.4
5 ⁵	4	15	92	21	50(66)	13.2
10	0	2	100	0	3(66)	13.1
15 ⁵	0	2	100	0	3(66)	13.1
20 ⁵	0	0	100	-	0(66)	13.0

¹V = Viable eggs; NV = Non-Viable eggs.

²% Viable = Percent viable eggs found in sample, compared to total number of eggs observed.

³% Red. = Percent of viable eggs noted to be reduced from number of viable eggs found at 1 hr operation.

⁴% Rec. = Percent of total number of eggs recovered to total theoretical number of eggs (illustrated in parentheses) that should be found assuming decrease in number of eggs is due only to dilution.

⁵These samples were neutralized, then refrigerated for 5 to 12 days before parasite analysis.

TABLE E-5. EFFECTS OF LIME STABILIZATION ON ASCARIS EGGS (FROM PILE DIRT OF WORMS) AND TOXOCARA EGGS (FROM DOG FECES) IN PRIMARY SLUDGE UNDER AEROBIC CONDITIONS AT AMBIENT TEMPERATURE

Time (days)	Ascaris					Toxocara					pH
	Eggs/50 ml		Percent Decrease Viable Eggs ²	Percent Viable ³	Percent Recovery of Total Eggs ⁴	Eggs/50 ml		Percent Decrease Viable Eggs ²	Percent Viable ³	Percent Recovery of Total Eggs ⁴	
	V	NV ¹				V	NV ¹				
No Lime Dosage											
0.04	0	1	-	0	1 (100)	0	6	-	0	6 (100)	7.5
5	0	0	-	-	0 (100)	0	3	-	0	3 (100)	7.4
10	0	5	-	0	5 (100)	0	7	-	0	7 (100)	5.1
15 ⁵	0	0	-	-	0 (100)	0	0	-	-	0 (100)	5.5
20 ⁵	0	0	-	-	0 (100)	0	0	-	-	0 (100)	6.2
100 mg of Lime (Ca(OH) ₂) Per Gram of Suspended Solids											
0.04	0	0	-	-	0 (100)	0	3	-	0	3 (100)	10.2
5	0	0	-	-	0 (100)	0	0	-	-	0 (100)	8.1
10	0	0	-	-	0 (100)	0	0	-	-	0 (100)	7.6
15 ⁵	0	0	-	-	0 (100)	0	0	-	-	0 (100)	7.4
20 ⁵	0	0	-	-	0 (100)	0	0	-	-	0 (100)	7.4
1000 mg of Lime (Ca(OH) ₂) Per Gram of Suspended Solids											
0.04	0	0	-	-	0 (100)	0	0	-	-	0 (100)	12.6
5	22	12	-	78	34 (100)	15	60	-	20	75 (100)	8.1
10	0	0	-	-	0 (100)	0	0	-	-	0 (100)	7.9
15 ⁵	0	0	-	-	0 (100)	0	0	-	-	0 (100)	8.0
20 ⁵	0	0	-	-	0 (100)	0	0	-	-	0 (100)	8.3
3000 mg of Lime (Ca(OH) ₂) Per Gram of Suspended Solids											
0.04	0	0	-	0	0 (100)	0	0	-	0	0 (100)	12.7
5	11	3	-	79	14 (100)	0	93	-	0	93 (100)	12.7
10	0	31	-	0	31 (100)	0	66	-	0	66 (100)	13.0
15 ⁵	0	23	-	0	23 (100)	0	74	-	0	74 (100)	13.1
20 ⁵	0	1	-	0	1 (100)	0	4	-	0	4 (100)	11.6

¹V = Viable Eggs, NV = Non-Viable Eggs.

²Percent noted to be reduced from number found in first sample.

³As compared to total number of eggs observed.

⁴Percent recovered to the total expected number (illustrated in parentheses) assuming decrease is due only to dilution.

⁵These samples were neutralized, then refrigerated for 5 to 12 days before parasite analysis.

TABLE E-6. EFFECTS OF TIME STABILIZATION ON ASCARIS EGGS (FROM THE SMALL INTESTINAL CONTENTS OF SWINE) IN AEROBICALLY DIGESTED SLUDGE MAINTAINED UNDER AEROBIC CONDITIONS AT AMBIENT TEMPERATURES AFTER LIME ADDITION

Time (days)	Digested at 28 th					pH	Digested at 35 th					pH
	Ascaris Eggs/50 ml ¹		Percent ² Decrease Viable Eggs	Percent ³ Viable	Percent ⁴ Recovery of Total Eggs		Ascaris Eggs/50 ml ¹		Percent ² Decrease Viable Eggs	Percent ³ Viable	Percent ⁴ Recovery of Total Eggs	
	V	NV					V	NV				
No Lime Dosage												
0.04	28	7	-	84	30 (162)	7.1	64	30	-	68	61 (152)	7.0
5 ⁵	15	4	61	79	12 (162)	9.6	40	14	38	74	35 (152)	8.1
10 ⁵	48	8	0	86	35 (162)	6.8	79	21	0	79	65 (152)	6.0
15	49	7	0	88	35 (162)	6.1	38	13	41	75	33 (152)	5.9
20	22	4	42	85	16 (162)	5.6	46	9	28	84	36 (152)	5.7
100 mg lime (Ca(OH) ₂) per Gram Suspended Solids												
0.04	13	2	-	87	9 (162)	8.7	52	18	-	74	45 (152)	10.1
5 ⁵	18	6	0	75	15 (162)	8.5	29	7	44	81	21 (152)	8.6
10 ⁵	34	5	0	87	24 (162)	8.4	48	13	8	79	40 (152)	8.5
15	42	4	0	91	28 (162)	8.8	66	17	0	80	54 (152)	8.8
20	32	9	0	78	25 (162)	8.1	12	8	77	60	13 (152)	9.1
1000 mg lime (Ca(OH) ₂) per Gram Suspended Solids ^b												
0.04	3	24	89	11	17 (162)	11.0	0	45	100	0	29 (152)	12.9
5 ⁵	2	4	93	33	4 (162)	12.7	0	7	100	0	5 (152)	12.8
10 ⁵	0	3	100	0	2 (162)	8.3	0	3	100	0	2 (152)	8.1
15	8	17	71	32	15 (162)	8.4	5	26	92	16	20 (152)	8.5
20	0	9	100	0	6 (162)	8.1	1	19	98	5	11 (152)	8.0
3000 mg lime (Ca(OH) ₂) per Gram suspended Solids ^b												
0.04	0	24	100	0	15 (162)	13.1	1	32	98	3	21 (152)	13.0
5 ⁵	0	2	100	0	1 (162)	13.2	0	0	100	0	0 (152)	12.5
10 ⁵	0	2	100	0	1 (162)	13.0	0	4	100	0	1 (152)	12.9
15	0	21	100	-	13 (162)	12.5	0	29	100	0	19 (152)	9.9
20	0	12	100	0	7 (162)	-	0	9	100	0	6 (152)	-

¹V = Viable Eggs; NV = Non-Viable Eggs.²Percent noted to be reduced from number found at one hour of operation.³As compared to total number of eggs observed.⁴Percent recovered to total expected number (illustrated in parentheses) assuming decrease is due only to dilution.⁵These samples were neutralized, then refrigerated for 5 to 12 days before parasite analysis.⁶Percent noted to be reduced from number found in no lime dosage at one hour of operation at specified temperature.

TABLE E-7. EFFECTS OF LIME STABILIZATION ON ASCARIS EGGS (FROM SMALL INTESTINAL CONTENTS OF SWINE) IN AEROBICALLY DIGESTED SLUDGES MAINTAINED UNDER ANAEROBIC CONDITIONS AT AMBIENT TEMPERATURES AFTER LIME ADDITION

Time (days)	Digested at 28°C						Digested at 35°C					
	Ascaris Eggs/50 ml ¹		Percent ² Decrease Viable Eggs	Percent ³ Viable	Percent ⁴ Recovery of Total Eggs	pH	Ascaris Eggs/50 ml ¹		Percent ² Decrease Viable Eggs	Percent ³ Viable	Percent ⁴ Recovery of Total Eggs	pH
	V	NV					V	NV				
No Lime Dosage												
0.04 ⁵	39	5	-	89	27 (162)	7.2	62	18	-	78	52 (144)	7.3
5 ⁵	29	5	26	85	21 (162)	6.7	38	7	39	84	29 (144)	6.7
10	42	8	0	84	31 (162)	7.4	93	16	0	85	71 (144)	7.5
15	47	2	0	96	30 (162)	8.0	44	8	29	85	34 (144)	8.0
20	47	7	0	87	33 (162)	7.2	58	15	6	79	47 (144)	7.1
100 mg Lime (Ca(OH) ₂) per Gram Suspended Solids												
0.04 ⁵	21	9	-	70	19 (162)	12.4	51	10	-	84	40 (144)	8.6
5 ⁵	10	14	52	72	15	12.4	53	20	0	73	47 (144)	7.5
10	3	29	86	10	20 (162)	12.2	66	8	0	89	78 (144)	7.8
15	30	12	0	71	26 (162)	11.9	62	13	0	83	79 (144)	8.0
20	0	3	100	0	2 (162)	11.5	83	7	0	92	50 (144)	7.7
1000 mg Lime (Ca(OH) ₂) per Gram Suspended Solids												
0.04 ⁵	15	14	-	52	18 (162)	13.3	27	28	-	79	36 (144)	13.1
5 ⁵	0	14	100	0	9 (162)	13.0	20	40	26	33	39 (144)	13.1
10	6	30	60	17	22 (162)	13.0	17	32	37	35	32 (144)	13.0
15	18	22	0	15	25 (162)	12.6	81	1	0	99	53 (144)	12.7
20	2	43	87	4	28 (162)	12.2	49	23	0	68	48 (144)	12.3
3000 mg Lime (Ca(OH) ₂) per Gram Suspended Solids												
0.04 ⁵	28	32	-	77	37 (162)	13.3	44	30	-	59	48 (144)	13.1
5 ⁵	1	13	96	7	9 (162)	13.2	3	29	93	9	21 (144)	13.0
10	2	8	93	20	6 (162)	13.1	1	6	98	14	5 (144)	13.1
15	19	18	32	51	23 (162)	12.6	40	18	9	69	38 (144)	12.8
20	2	27	93	7	18 (162)	12.2	48	23	0	68	46 (144)	12.3

¹V = Viable eggs; NV = non-viable eggs.

²Percent noted to be reduced from number found at one hour of operation.

³As compared to total number of eggs observed.

⁴Percent recovered to total expected number (illustrated in parentheses) assuming decrease is due only to dilution.

⁵These samples were neutralized, then refrigerated for 5 to 12 days before parasite analysis.

TABLE E-8. EFFECTS OF AMMONIA ON ASCARIS EGGS (FROM THE SMALL INTESTINAL CONTENTS OF SWINE) IN LIME STABILIZED-AEROBICALLY DIGESTED SLUDGE (3000 mg LIME (Ca(OH)₂) PER GRAM SUSPENDED SOLIDS UNDER ANALEROBIC CONDITIONS AT AMBIENT TEMPERATURE)

Time (days)	Digested at 28 ⁰ C							Digested at 35 ⁰ C						
	Ascaris Eggs/50 ml ¹		Percent ² Decrease Viable Eggs	Percent Viable ³	Percent ⁴ Recovery of Total Eggs	pH	Ammonia Concen- tration	Ascaris Eggs/50 ml ¹		Percent ² Decrease Viable Eggs	Percent Viable ³	Percent ⁴ Recovery of Total Eggs	pH	Ammonia Concen- tration
	V	NV						V	NV					
No Ammonia Dosage														
0.04	28	32	-	83	37 (162)	13.3	25	81	1	-	99	53 (144)	13.1	28
5	1	13	96	99	9 (162)	13.2	25	3	29	96	9	21 (144)	13.0	28
10	2	8	93	99	6 (162)	13.1	25	1	6	99	14	5 (144)	13.1	28
15	10	18	100	100	7 (162)	12.6	25	44	8	51	69	38 (144)	12.8	28
50 mg Ammonia Sulfate per Gram Suspended Solids														
0.04	69	0	-	100	43 (162)	13.1	147	60	0	-	100	39 (144)	13.2	133
5	7	18	90	28	15 (162)	13.1	147	16	15	73	52	20 (144)	13.2	133
10	1	6	99	14	4 (162)	13.1	147	1	7	98	13	5 (144)	13.2	133
15	0	11	100	0	5 (162)	12.7	147	0	46	100	0	30 (144)	12.7	133
500 mg Ammonia Sulfate per Gram Suspended Solids														
0.04	56	15	-	79	44 (162)	13.1	1,162	37	0	-	100	24 (144)	13.2	1,050
5	1	10	98	9	7 (162)	13.0	1,162	17	12	54	59	19 (144)	13.0	1,050
10	0	3	100	0	2 (162)	13.0	1,162	0	9	100	0	6 (144)	13.0	1,050
15	0	8	100	0	2 (162)	12.7	1,162	0	21	100	0	14 (144)	12.8	1,050
5000 mg Ammonia Sulfate per Gram Suspended Solids														
0.04	66	1	-	99	41 (162)	13.3	11,620	66	4	-	94	45 (144)	13.2	10,640
5	0	13	100	0	8 (162)	13.2	11,620	0	39	100	0	25 (144)	13.1	10,640
10	0	26	100	0	16 (162)	13.1	11,620	0	37	100	0	24 (144)	13.0	10,640
15	0	4	100	0	26 (162)	12.7	11,620	0	60	100	0	39 (144)	12.8	10,640

¹V = viable eggs, NV = non-viable eggs (viability determined by cultivation).

²Percent noted to be reduced from number found at one hour of operation

³As compared to total number of eggs observed

⁴Percent recovered to total expected number (illustrated in parentheses) assuming decrease is due only to dilution

TABLE E-9. EFFECTS OF AMMONIA ON ASCARIS EGGS (FROM THE SMALL INTESTINAL CONTENTS OF SWINE)
AND TOXOCARA EGGS (FROM THE FECES OF DOGS) IN 35°C ANAEROBICALLY DIGESTED SLUDGE
UNDER ANAEROBIC CONDITIONS AT AMBIENT TEMPERATURE

Time (days)	pH	[NH ₄] ¹ mg/l	Ascaris		Percent Decrease V Eggs ³	Σ V ⁴	Σ Total ⁵ Recovery	Toxocara		Percent Decrease V Eggs ³	Σ V ⁴	Σ Total Recovery
			Eggs/50 ml ² V	NV				Eggs/50 ml ² V	NV			
NO AMMONIA DOSAGE												
0	12	330	7	3	-	70	34	6	6	-	50	34
5	12	330	15	1	0	94	55	17	10	0	63	77
50 mg AMMONIA SULFATE PER GRAM SUSPENDED SOLIDS												
0	12	462	8	2	-	80	34	13	10	-	57	66
5	12	462	8	1	0	89	31	9	13	31	41	63
500 mg AMMONIA SULFATE PER GRAM SUSPENDED SOLIDS												
0	12	1,638	11	1	-	92	41	11	6	-	65	49
5	12	1,638	23	2	0	92	86	12	19	0	39	89
5000 mg AMMONIA SULFATE PER GRAM SUSPENDED SOLIDS												
0	12	12,880	8	1	-	89	31	9	9	-	50	51
5	12	12,880	14	10	0	58	83	12	18	0	40	86

¹[NH₄]⁺mg/l = concentration of free ammonia in mg/l as nitrogen.

²V = viable eggs; NV = non-viable eggs (viability determined by cultivation).

³Percent noted to be reduced from number found at beginning.

⁴As compared to total number of eggs observed.

⁵Percent recovered to total expected number of eggs.