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Project Summary

Parasites in Southern Sludges and Disinfection by Standard Sludge Treatment

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The objectives of this study 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 parasite inactivation by lime treatment of sludges seeded with intestinal parasites, (3) measure the mass balance of helminth eggs through various processes in a municipal wastewater treatment plant, and (4) assess, on the basis of laboratory and field data, standard sewage sludge treatment processes for their effectiveness in inactivating 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 using parasitologic techniques developed for this study. Viable eggs of *Ascaris* and *Toxocara* were recovered at least once from every plant and viable eggs of *Trichuris vulpis* and *Trichuris 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. Depending upon the parasite, the inactivation of parasites during sewage treatment fluctuated from season to season, but, in general, most were inactivated in the summer. Laboratory studies verified the results of previous investigations indicating that destruction of resistant parasite eggs is primarily due to temperature (heat) and not to a specific digestion process. Very large lime doses were required for the inactivation of viable *Ascaris* in sludges and results were not always consistent. Lime treatment thus appears to be an expensive and unreliable treatment for *Ascaris* inactivation. 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.

An important finding of this study is the poor suitability of *Ascaris* eggs taken from the uteri of gravid female worms as indicators of the characteristics of *Ascaris* eggs discharged in the feces of the host. The eggs removed from the gravid females are not as resistant to adverse factors as eggs that have undergone a hardening process in the intestines of the host and been recovered from the feces.

This Project Summary was developed by EPA's Municipal Environmental Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

In the United States, land disposal of sewage sludge has been practiced with care because of controversies over possible health and nuisance problems. Pathogens are known to survive conventional sludge stabilization and dewatering processes including the use of chemicals in these conventional processes. Of the three general types of pathogenic organisms—bacteria, viruses, and parasites—found in sewage sludges, certain parasites are known to be the most resistant to conventional sludge treatment processes. Also, parasites are the least studied of the pathogenic organisms found in sewage sludges. It was the general purpose of this study to investigate the types and densities of parasites in sewage sludges in the southern United States where both humidity and temperature favor parasite survival. It was also the purpose of this study to investigate, through field data and laboratory studies, the effectiveness of conventional sludge stabilization and select new processes for inactivating parasites in sewage sludges.

Research Program

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 effect of abattoir wastes on the density of parasites in sludge; and 4) other factors affecting the prevalence and persistence of parasites in sludges. Laboratory studies investigated the effect of selected sludge treatment processes on parasite eggs and cysts found in sewage sludge. The treatment processes investigated were aerobic and anaerobic digestion, lime stabilization, ammonification, sonication, and various combinations of these processes.

Results

Field Studies

Parasitological Findings

Sludge samples collected during each of the four seasons from 27 municipal wastewater treatment plants located in Alabama, Florida, Mississippi, Louisiana, and Texas were examined for the presence and density of the resistant stages of human and animal parasites using parasitologic techniques developed for this study. The selection of the wastewater treatment plants for sludges was made on the basis of the method of wastewater treatment used, size of the treatment plant, type of population served, and geographic and climatologic features of the region.

The results of the parasitologic examination are shown in Tables 1 through 3. Many of the eggs or cysts of parasites found in sludges were identified only as to genus or type 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* (human roundworm) and *A. lumbricoides* var *suum* (pig roundworm) are virtually indistinguishable and, consequently, when *Ascaris* eggs are found, it could require examination with an electron microscope to distinguish between the two species. The probable identity of each type of helminth egg and protozoan cyst found in the sludges is shown in Table 1.

Ascaris, Toxocara, Trichuris trichiura, and Trichuris vulpis were the parasites most commonly found in the sludges (see Table 2). 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 3 are listed other parasites, either viable or non-viable, found in the sludges. Of these parasites, *Hymenolepis diminuta* was most frequently found; its eggs were observed 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 sewage systems and treatment plants. Other parasite eggs that are more 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.

Densities of Parasites in Sludges

The total number of parasite eggs recovered from the digested sludge samples ranged from 0 to more than 230,000 eggs/kg dry weight of sludge, depending on the source of sludge and season of the year. The average number of total parasite eggs was approximately 14,000/kg dry weight of sludge. The percentage of the total parasite eggs in the sludge samples that were viable ranged from 0% to 100%, but was generally greater than 45% for primary sludge and 69% for treated sludge (see Table 4). Primary and secondary undigested sludge samples were found to contain in order of decreasing average densities: 9,700 *Ascaris* spp. eggs, 1,200 *Toxocara* spp. eggs, 800 *T. trichiura* eggs, and 600 *T. vulpis* eggs/kg dry weight of sample. The average numbers of these parasites in stabilized sludge samples were: 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. Densities of these four most prevalent parasites fluctuated greatly. The standard deviation of their densities is greater than the observed averages and the ranges are from zero to 10 times the average. Other parasite eggs and cysts were observed in the sludge samples, but in low concentration.

The Influence of Abattoirs on Parasite Concentration

Unusually high levels of *Ascaris* eggs were found in the sludges of one wastewater treatment plant. A detailed study revealed that an abattoir in the community processed large numbers of swine and that the wastes from this abattoir entered the municipal sewer system. In Table 5, the levels of parasite eggs in the wastewater treatment sludges receiving the abattoir wastes are compared with 6 other wastewater treatment plants of similar size in the same geographic area. In the plant receiving abattoir wastes, 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 7,900 *Ascaris* eggs/kg was recovered. The *Ascaris* eggs in the treatment plant receiving the abattoir wastes were undoubtedly mostly *A. suum* eggs that came from infected swine. It is interesting that the level of the *T. trichiura*-like eggs was nearly the same in each plant. This would indicate that very few, if any, of

Table 1. *Parasites Found in Sludge Samples from 27 Municipal Plants in Southern United States*

Parasite Found	*Probable Identity*	*Definitive Host*
Ascaris *eggs*	Ascaris lumbricoides[1] Ascaris suum[1]	*Humans* *Pigs*
Toxocara *eggs*	Toxocara canis[2] Toxocara cati[2]	*Dogs* *Cats*
Trichuris trichiura	Trichuris trichiura Trichuris suis[3]	*Humans* *Pigs*
Trichuris vulpis *eggs*	Trichuris vulpis	*Dogs*
Toxascaris-*like eggs*	Toxascaris leonina	*Dogs and Cats*
Ascaridia-*like eggs*	Ascaridia galli Heterakis gallinae	*Domestic poultry* *Domestic poultry*
Trichosomoides-*like eggs*	Trichosomoides crassicauda Anatrichosoma buccalis	*Rats* *Opossums*
Cruzia-*like eggs*	Cruzia americana	*Opossums*
Capillaria *spp. eggs* *(3 or more types)*	Capillaria hepatica Capillaria gastrica Capillaria *spp.* Capillaria *spp.* Capillaria *spp.*	*Rats* *Rats* *Domestic poultry* *Wild birds* *Wild mammals (opossums, racoons, etc.)*
Hymenolepis diminuta *eggs*	Hymenolepis diminuta	*Rats*
Hymenolepis nana *eggs*	Hymenolepis nana	*Humans and rodents*
Hymenolepis *sp. eggs*	Hymenolepis *spp.* *(poss. more than one species)*	*Domestic and/or wild birds*
Taenia *sp. eggs*	Taenia saginata[4] Taenia pisiformis[4] Hydratigera taeniaeformis[4]	*Humans* *Cats* *Dogs*
Acanthocephalan eggs	Macracanthorhynchus hirudinaceus	*Pigs*
Entamoeba coli-*like eggs*	Entamoeba *coli*[5] Entamoeba *spp.*	*Humans* *Rodents, etc.*
Giardia *cysts*	Giardia lamblia Giardia *spp.*	*Humans* *Dogs, cats, mammals*
Coccidia *oocysts*	Isospora *spp.* Eimeria *spp.*	*Dogs, cats* *Domestic and wild birds, mammals*

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

these eggs were those of *T. suis*, the swine whipworm.

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) were not very effective in destroying parasite eggs (see Figure 1). The number of viable *Ascaris* and *Toxocara* per unit dry weight of sludge actually increased during these processes due to the loss of dry mass of sludge solids that occurs in the digestion processes. The concentration processes of vacuum filtration and centrifugation appear to have removed or destroyed eggs, but due to the low number of samples analyzed and variable nature of the effect, this cannot be confirmed. The use of drying beds, however, is consistently very effective for destroying parasites in sludges.

In the field investigation, data were collected on both raw (undigested) sludges and on sludges stabilized by either aerobic digestion or anaerobic digestion under ambient or mesophilic temperatures followed by dewatering on drying beds. These data are shown in Table 6. During the winter and fall, parasite inactivation tended to be most

Table 2. *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*
Ascaris	*17*[2]*/25*[3]*/26*[4]	*22/25/26*	*14/26/27*	*14/25/25*	*26/27/27*
Toxocara	*11/22/24*	*17/27/27*	*9/24/24*	*9/23/25*	*23/27/27*
Trichuris trichiura	*6/10/16*	*8/12/18*	*7/10/19*	*6/10/16*	*12/15/26*
Trichuris vulpis	*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 3. *Miscellaneous Parasites Found in Sludges from 27 Municipal Treatment Plants Sampled*

Parasite	*No. of plants in which found*
Toxascaris leonina *eggs*	*2*
Ascaridia-*like eggs*	*7*
Cruzia-*like eggs*	*1*
Trichosomoides-*like eggs*	*7*
Capillaria *eggs (shells with pits)*	*7*
Capillaria *eggs (shell with striations)*	*11*
Hymenolepis diminuta *eggs*	*23*
Hymenolepis nana *eggs*	*6*
Taenia *sp. eggs*	*1*
Acanthocephalan eggs	*1*
Entamoeba coli-*like cysts*	*23*
Giardia *cysts*	*9*
Coccidia *oocysts*	*6*

variant. Except for *Toxocara*, the densities of all viable parasite eggs were reduced more in the summer and spring than in the fall and winter. Table 6 indicates the influence of anaerobic or aerobic digestion on drying bed treatment for parasite eggs. The percent reduction of viable eggs of four predominant parasites with respect to the drying bed process was generally not influenced by either aerobic or anaerobic stabilization. However, some reduction in effectiveness during the fall and winter was noted with anaerobically digested sludges, yet with aerobically digested sludges, a seasonal fluctuation was noted only with *T. trichiura*.

A correlation was found between the density of inactivated parasites and moisture contents in drying bed sludges. Figure 2 shows the correlation of the logarithm of the numbers of viable *Ascaris* eggs in raw versus drying bed sludge in the same plants (grouped by the moisture content of the drying bed sludges). The inactivation of viable parasite eggs in the raw sludges increases with decreasing moisture content of the drying bed sludges. The densities of viable *Ascaris* and *Toxocara* eggs in the drying bed sludges as related to the sludge moisture content was analyzed for each season. Table 7 shows that lowest moisture levels at which all *Ascaris* or *Toxocara* eggs were inactivated was 5% in the fall, 7% in the

Table 4. *Parasite Concentrations in Primary and Secondary Sludge as Compared to Treated Sludge*

Parasite	*Nature of Sludge*[1]	*Number of Viable and Non-Viable Eggs/kg Dry Weight of Sample*			*Percent Viable Eggs*
		Average[2]	*Standard Deviation*	*Range*	
Ascaris *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*
Trichuris trichiura *(human whipworm)*	*Primary and Secondary*	*800*	*2,900*	*26,000 - 0*	*50*
	Treated	*2,600*	*9,800*	*84,000 - 0*	*48*
Trichuris vulpis *(dog whipworm)*	*Primary and Secondary*	*600*	*1,000*	*5,700 - 0*	*90*
	Treated	*700*	*1,300*	*10,500 - 0*	*64*
Toxocara *spp. (dog and cat roundworm)*	*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.*

Table 5. *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
Ascaris *spp.* *(human and pig roundworms)*	*Domestic*[2]	*7,900*	*6*
	Abattoir[3]	*81,800*	*1*
Trichuris trichiura *or* Trichuris suis *(human or pig whipworms)*	*Domestic*	*1,500*	*6*
	Abattoir	*1,600*	*1*
Toxocara *spp.* *(dog and cat roundworms)*	*Domestic*	*1,800*	*6*
	Abattoir	*500*	*1*

[1]*Numbers rounded off to nearest 100.*
[2]*Domestic plants found in the geographic area.*
[3]*Treatment plant in the geographic area receiving waste from large swine slaughter and packing houses.*

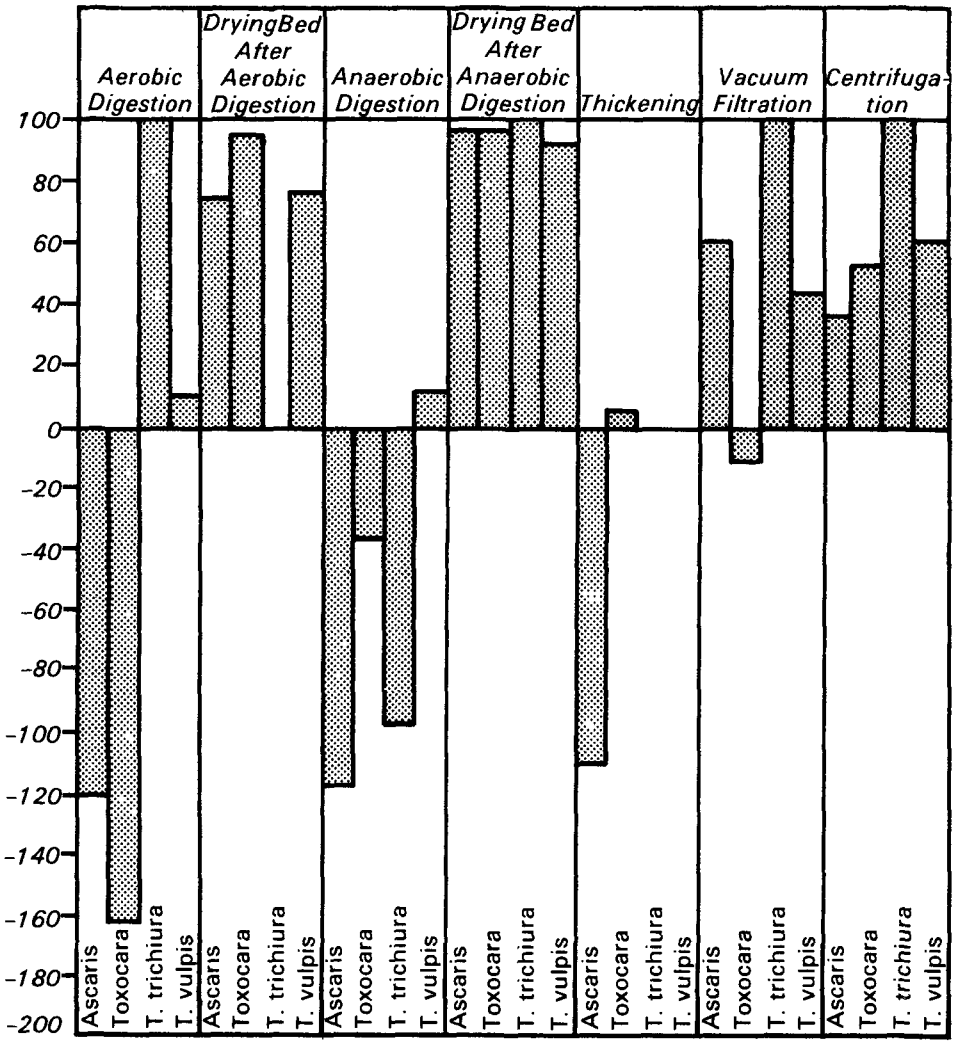

Figure 1. *Average percent reduction of viable parasite eggs by unit process. (Negative values indicate that densities of parasites increased after the process.)*

winter, 8% in the spring, and 15% in the summer. Evidently, both temperature and reduction in moisture content play a part in the inactivation of these parasites.

Mass Balance of *Ascaris* Eggs Through a Secondary Wastewater Treatment System

A mass balance of parasite eggs through each of the major unit processes was conducted in a municipal wastewater treatment plant which had high densities of parasites in the raw wastewater because of the contribution of wastes from an abattoir. Major unit processes consisted of contact stabilization, aerobic sludge stabilization, and sludge drying beds. Concentrations of *Ascaris* eggs were the highest prior to peak flows during early morning and late afternoon; whereas general municipal constituents fluctuated as expected. A possible explanation for this phenomenon 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 removed (concentrated in the sludge) 91 to 98% of the *Ascaris* eggs present in the contact stabilizer effluent. However, 5 to 51 viable *Ascaris* eggs per liter of effluent were being discharged into a receiving creek. The percent removal of parasite eggs decreased as flow rate increased and clarifier removal efficiency decreased.

Except for sludge wasting, the *Ascaris* eggs removed by secondary clarification were recycled in the return sludge and added to the influent sewage, thus

Table 6. *Percent Reduction of Viable Parasite Eggs by Total Sludge Treatment Processes*[1]

Process (Period)	Ascaris	Toxocara	T. trichiura	T. vulpis
Total	*67/76/69*[2]	*91/28/74*	*39/118/26*	*44/87/58*
Fall	*58/101/16*	*82/39/16*	*31/87/7*	*(1)*[3]*/135/8*
Winter	*36/108/17*	*89/37/22*	*(26)/196/7*	*7/108/16*
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 parenthesis indicate percent increase.*

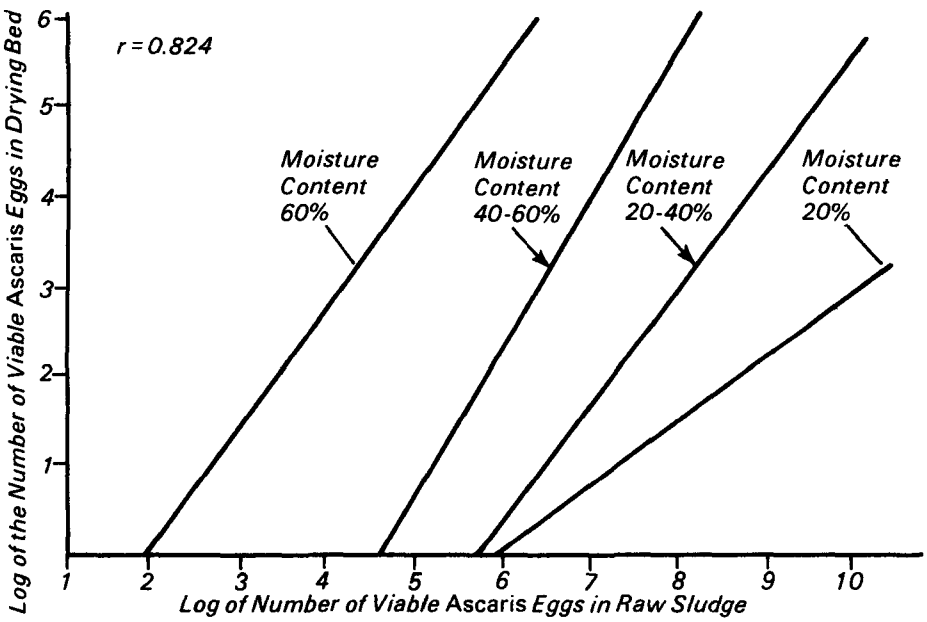

Figure 2. *Plot of log of number of viable* Ascaris *eggs in raw sludge grouped by moisture content of drying bed sludges.*

effectively concentrating the eggs in the contact stabilized and reaerated sludge. Therefore, it would appear that the processes of contact stabilization, activated sludge, or extended aeration tend to maintain a uniform level of parasite eggs in the sludges of the treatment plant.

Laboratory Studies

Laboratory studies on selected wastewater sludges were conducted to determine the factors involved in the inactivation of parasite eggs and cysts in sewage sludges. Bench scale results of semi-continuous aerobic (ten day hydraulic retention time) or anaerobic sludge digestion (fifteen day hydraulic retention time), lime stabilization, ammonification, sonication, and combinations of the above processes for the destruction of *Ascaris suum* and *Toxocaris canis* eggs in sludges are briefly as follows:

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 treatment of sludges, pretreated by aerobic digestion at 28 °C and 35 °C to produce maximum embryonation (and thus maximum sensitivity to environmental factors), did not produce consistent inactivation of *Ascaris* eggs. The sludges were lime treated and then held under aerobic or anaerobic conditions. With one exception, inactivation increased with increasing contact time (up to 20 days) and increasing lime dose, although 3,000 mg $Ca(OH)_2$/gram sludge solids was required for essentially complete inactivation. The exception, 35 °C aerobically digested sludge stored under anaerobic conditions, showed poor and erratic inactivations at the various storage times and lime doses. Lime treatment thus appears to be an expensive and unreliable method for *Ascaris* inactivation.
4) The results of the ammonification studies were inconclusive. In aerobically digested sludges, the viable *Ascaris* eggs densities were reduced 95% within 5 days even in the control (no ammonia added). The anomalously high reductions with the control casts doubt on this set of data. In the anaerobically digested sludges, no reduction in *Ascaris* viability was observed at any dosage of ammonia up to 5,000 mg of ammonia sulfate per gram of suspended solids.
5) Ultrasonication was effective in destroying *Toxocara* eggs at 49kHz within a 6 minute exposure, but ultrasonication was not effective in destroying *Ascaris* eggs under these same conditions. The test samples consisted of a suspension of approximately 10,000 eggs of

Table 7. *The Relationship Between the Viability of Ascaris* and *Toxocara Eggs in Drying Bed Sludges and the Moisture Content of the Sludge, in Different Seasons*

Season	Number of Drying Bed Samples Analyzed	Number of Samples With No Viable Eggs	Lowest Moisture Contents Below Which No Viable Eggs Observed	Number of Samples With No Viable Eggs At or Below Lowest Moisture Contents	Number of Samples With Viable Eggs
			For Both Ascaris *and* Toxocara *Eggs*		
Fall	*24*	*7*	*5%*	*1*	*11*
Winter	*22*	*4*	*7%*	*1*	*18*
Spring	*22*	*12*	*8%*	*8*	*10*
Summer	*21*	*14*	*15%*	*8*	*7*
			For Only Ascaris *Eggs*		
Fall	*21*	*7*	*5%*	*1*	*14*
Winter	*22*	*5*	*7%*	*1*	*17*
Spring	*22*	*12*	*8%*	*8*	*10*
Summer	*21*	*14*	*15%*	*8*	*7*
			For Only Toxocara *Eggs*		
Fall	*24*	*12*	*5%*	*1*	*12*
Winter	*22*	*12*	*21%*	*3*	*10*
Spring	*22*	*17*	*21%*	*11*	*5*
Summer	*21*	*18*	*20%*	*10*	*3*

the respective parasite in 100 ml of water.

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***Gerald Stern** is the EPA Project Officer (see below).*
The complete report, entitled "Parasites in Southern Sludges and Disinfection by Standard Sludge Treatment," (Order No. PB 82-102 344; Cost: $17.00, subject to change) will be available only from:
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