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Project Summary

Survival and Transport of Pathogens in Sludge-Amended Soil: A Critical Literature Review

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A study was undertaken to critically review available information on the survival and transport of pathogens from municipal wastewater sludges applied to land. Unfortunately, the amount of quantitative, comparable data related to pathogen behavior in sludge-amended soils is extremely limited. Most available data are restricted to *Salmonella* and indicator bacteria.

In general, *Salmonella* showed a 90% (T_{90}) reduction within 3 weeks in sludge-amended soils. In warm climates, inactivation of viruses near the surface was quite rapid, with a median T_{90} of 3 days. However, at low temperatures, T_{90} values of approximately 30 days were observed for viruses. Maximal parasite survival, as determined by *Ascaris* ova recovery, was relatively long near the surface, with a median T_{90} of 77 days.

Extremely limited vertical movement of some pathogens may be anticipated in sludge-amended soils. Although monitoring at sludge application sites has not revealed that sludge amendment affects the bacterial quality of groundwater, limited transport of indicator bacteria to depths up to 180 cm has been reported. Under field conditions including exposure to natural rainfall, virtually no viruses have been detected in soil-water percolates. Available literature strongly favors the contention that parasitic ova are retained at the point sludge is introduced to the soil. Finally, insufficient data are available for adequate modeling of pathogen survival and transport in sludge-amended soils.

This Project Summary was developed by EPA's Water Engineering 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

An integral part of almost any wastewater treatment plant is the sludge management system. Residual solids are produced in nearly every unit process associated with conventional wastewater treatment. Approximately 6.5 million dry tons of municipal wastewater sludges are generated annually in the United States. By the year 2000, the quantity of municipal wastewater sludge produced is projected to almost double. Sludge management currently accounts for approximately half of the cost of wastewater treatment. Thus a major goal is to reduce the costs of sludge handling. Equally important is to reduce to an acceptable level the risks to public health, safety, and welfare that arise from and are otherwise associated with sludge disposal.

Among several disposal alternatives, land application of sludge is increasing in popularity. Indeed, there is every reason to believe that the practice will expand at a greater rate in the years to come. The presence of infectious microorganisms in sludges may, however, place certain constraints on their use on land.

The concentration of pathogens in wastewater and thus in wastewater sludges is influenced by a number of factors, including the age and health of the contributing population, population density, sanitary habits, and the season of the year. Microorganisms of public health concern are generally classified into three broad categories: bacteria, viruses, and parasites. Parasites are often further differentiated into helminths and protozoans. Hundreds of organisms fall into these categories and may be present in domestic wastewaters.

A wide variety of disease-causing microorganisms known to be transmitted by the fecal-oral route may potentially be transmitted through environmental exposure. A more focused list of microbial agents can be prepared, however, with the application of additional criteria such as demonstrable presence in wastewaters or sludges and/or documented environmental transmission of disease. Table 1 provides such a listing. Although some of the organisms listed are overt pathogens, reports of their occurrence in wastewater and sludges in the United States are quite rare. However, advances in microbiological and medical sciences may identify additional pathogenic organisms linked to environmental disease transmission.

Wastewater treatment affects the various organism types in different ways. In general, microbial segregation occurs during conventional wastewater treatment. Bacteria, viruses, and some parasitic cysts tend to become associated with the sludge component, as do the heavier eggs of certain parasites such as *Ascaris*. Conventional sludge treatment processes can reduce the levels of sludge-associated pathogens. In the absence of extensive treatment, however, wastewater sludges will contain measurable concentrations of these microorganisms. Thus from a public health standpoint, applying wastewater sludge to land needs regulation in regard to the pathogens known to be present in these sludges.

Interim regulations relating to sludge treatment and disposal have been developed and were published in 40 CFR Part 257. These current regulations are based on the expected operational performance of specific unit processes and on the absence of health effects directly related to land application practices. Ultimately, however, regulations should be founded on both a complete understanding of the fate and transport of pathogens in sludge-amended soils and on the epidemiological implications associated with the numbers of organisms to which humans are subjected as a result of these practices. To facilitate the development of scientifically based regulations, a critical review was made of available information on the survival and movement of pathogens from municipal wastewater sludges applied to land.

Methods

Acquisition of Literature

Extensive literature searches were conducted, and a significant number of documents were accumulated from a variety of sources. The search for relevant documents was carried out in four steps: primary (database) searches, secondary searches, author contacts, and manual searches.

A total of 12 primary literature databases were searched. This step resulted in the acquisition of a total of 819 titles and abstracts. After they were read and duplication was eliminated, 95 unique documents were identified for hardcopy acquisition.

The secondary literature searches were begun when the hard copies of documents from the primary searches were available for review. All references in the primary documents were considered possible secondary sources. Furthermore, each document from this secondary search represented possibilities for the identification of additional literature sources.

Contacts were made with authors identified as major contributors to the literature obtained during the primary and secondary searches. In addition, personal contacts were made at a number of national meetings. These efforts proved fruitful, as they obtained a number of obscure and unpublished documents of value to the study.

Manual searches were made of selected current science and engineering journal issues. The journals selected for manual searches were those that yielded documents relevant to the study through the primary and secondary literature searches.

Guidelines for Literature Evaluation

The literature review encountered a broad spectrum of studies ranging from investigations employing exogenously added organisms to monitoring of indigenous organisms at field sites. The

Table 1. *Organisms of Major Concern in Land Application of Municipal Wastewater and Sludges*

Group	Name of Organism	Primary Disease	Remarks
Bacteria	Legionella pneumophila	*Acute respiratory disease*	*Aerosol transmission documented, but no cases linked to wastewater exposure to date*
	Salmonella *sp.*	*Gastroenteritis, typhoid and paratyphoid fever*	
	Shigella *sp.*	*Bacillary dysentery*	*Overt pathogens but low probability of occurrence in wastewater in the United States*
	Vibrio cholerae	*Cholera*	
Viruses	*Hepatitis A virus*	*Infectious hepatitis*	*Documented waterborne transmission*
	Non-A, Non-B hepatitis	*Hepatitis*	*Preliminary evidence for waterborne transmission*
	Norwalk-like agents	*Gastroenteritis*	*Documented waterborne transmission*
	Rotavirus	*Gastroenteritis*	*Documented waterborne transmission*
Helminths	Ascaris *sp.*	*Ascariasis*	
Protozoans	Giardia lamblia	*Giardiasis (Gastroenteritis)*	*Documented waterborne transmission*

challenge posed by such diverse experimental conditions was to qualify the data within a common framework, reflecting (insofar as possible) expected responses in natural systems. To ensure a relatively unbiased appraisal of existing literature, guidelines were set to critically evaluate both laboratory and field studies before beginning the review of individual reports.

For example, attention was focused on the appropriateness of both sampling and analytical procedures for the recovery and identification of specific organisms. The adequacy of the experimental design was evaluated with regard to the number and frequency of samples collected as well as the control procedures used. From a regulatory and design standpoint, the collection of supporting data during the course of experimentation or monitoring could provide valuable information; thus particular attention was given to ancillary data that might affect pathogen survival or transport or both. Specifically, the collection of environmental data in the areas of temperature, rainfall, and various soil parameters was deemed important.

Finally, to provide a common framework within which organism survival could be addressed, simple inactivation values representing 90% (T_{90}) and 99% (T_{99}) dieaway were graphically determined. For this purpose, minimal criteria were established for using published data: initial monitoring of amended soil must have occurred within 2 weeks of sludge application, and a minimum of three positive, quantitative recoveries must have been recorded over a consecutive monitoring period. In addition, there must have been no data extrapolation beyond the actual sampling period.

Results and Discussion

In considering the various studies detailing the survival and transport of microorganisms in sludge-amended soils, the limitations influencing quantitative results in such systems must be recognized. Perhaps the most important problem in evaluating the behavior of microbes in sludges and soils is the methods used for organism recovery. Though standard methods for detecting indicator bacteria in water and wastewater have been widely applied in soil systems, these bacterial groups are not normally associated with human disease. The recovery and enumeration of bacterial pathogens, viruses, and parasites often require elaborate procedures involving a high degree of technical competence and experience. In addition, the factors affecting organism recovery from sludge and soil systems are not well understood. Furthermore, the recovery of viable bacteria, viruses, and parasites is limited by the volume of sample that can be analyzed, thus imposing, in some instances, a restrictive sensitivity limit on organism detection.

Survival of Microorganisms in Sludge-Amended Soils

Factors frequently cited as affecting microbial survival in soil include soil moisture content, temperature, pH, sunlight, organic matter, and antagonistic soil microflora. Microbial pathogens introduced into the soil by sludge amendment will be influenced by these factors. However, the nature of the sludge-amended soil environment may moderate soil conditions in ways that could affect the survival of microorganisms. For example, sludge application may dramatically increase the organic content, nutrient content, and moisture-retention capability of sandy soils. In addition, soil pH could be influenced by added sludge or management practices such as liming. Even soil temperature could be affected by the surface application of sludge. Though the interplay of these factors in sludge-amended soil may favor organism survival in some cases, more rapid pathogen inactivation may occur in other situations.

As shown in Table 2, T_{90} values for *Salmonella* survival in sludge-amended soil fall within the range of 3 to 61 days, with median values of 12 and 8 days for soil depths of 0 to 5 cm and 5 to 15 cm, respectively. A closer review of selected studies reveals a seasonal trend of bacterial inactivation. When salmonellae in sludge-amended soils were subjected to winter conditions, T_{90} values of 12 to 15, 17, and 22 to 61 days have been estimated in three published field studies. Similarly, at a temperature of 12°C, *Salmonella* sp. were observed to decay with a T_{90} of 8 to 11 days in a controlled laboratory study. During summer exposure, a much more rapid inactivation of indigenous salmonellae has been observed with T_{90} values of 4 and 6 days in two separate field studies. Experiments conducted during warm growing seasons with laboratory-grown strains of *Salmonella* have resulted in T_{90} values of 6 and 10 days in an Ohio study and 14 days in a Norway study. Hence studies with both indigenous and seeded salmonellae are in relatively good agreement, showing (with one exception) a 90% bacterial reduction within 3 weeks of sludge application.

The exceptionally long survival times often cited for *Salmonella* persistence actually arise from seeded studies in which high levels of bacteria ranging from 10^6 to 10^{10}/L were added to sludge before land application. Under these conditions, and assuming a maximum T_{99} of 45 days, persistence times in excess of 5 months could be anticipated. If growth or regrowth of seeded organisms occurs, this survival time could be substantially longer. On the other hand, indigenous salmonellae at actual field sites have generally persisted at low levels for less than 2 months, although a few positive recoveries have been reported as long as 3 to 5 months after sludge application.

Most published literature documents the behavior of indicator bacteria in sludge-amended soils. With the exception of one study, 90% of the fecal coliforms could not be recovered within 6 weeks of sludge application. A 90% loss of fecal streptococci occurred with 4 weeks. Although the number of studies is more limited, total coliform bacteria displayed significantly slower inactivation rates, with T_{90} values generally twice those of the other bacterial indicator groups (Table 2). Note, however, that T_{90} values for total coliforms are polarized, with most values ranging from 14 to 42 days and a second group ranging from 129 to 172 days. An evaluation of the overall survival results of these groups of bacteria reveals a dichotomy with the seasonal survival of coliform bacteria as reported by two research groups working in the Pacific Northwest region of the United States. Although the actual T_{90} values were dramatically different, both studies observed longer survival times during warmer months than during cooler months. Coliform regrowth is the most likely explanation of these findings. Indeed, regrowth of coliform bacteria in the spring following a decrease in levels during the winter has been reported. These results highlight the difficulty in evaluating bacterial inactivation when organisms are capable of replication. Interpretation of data for indicator bacteria is further complicated by the fact that several bacterial species, including unique soil microflora, may be recovered by the analytical procedures used. These bacterial populations do not necessarily share the same inactivation or regrowth characteristics.

Table 2. Summary of Microorganism Survival in Sludge Applied to Soil

Bacteria	Depth (cm)	Die-off—T_{90} (days) Minimum	Maximum	Median	Observations	Die-off—T_{99} (days) Minimum	Maximum	Median	Observations
Salmonella	0– 5	6	61	12	10	11	45	22	8
	5–15	3	22	8	17	7	45	18	15
Fecal streptococci	0– 5	7	28	14	9	14	63	24	8
	5–15	12	30	20	11	30	60	40	10
Fecal coliforms	0– 5	7	84	25	19	12	165	53	16
	5–15	4	49	13	12	9	90	32	11
Total coliforms	0– 5	16	172	85	6	28	350	155	4
	5–15	9	70	17	12	18	40	32	9
Viruses	0– 5	<1	30	3	9	2	52	6	6
	5–15	12	56	30	4	60	100	60	3
Parasites	0– 5	17	270	77	11	68	500	81	5
	5–15	Data unavailable				Data unavailable			

Survival of viruses in soils is influenced by many of the same parameters described for bacteria. The effect of temperature on the survival of viruses is well documented: lower temperatures favor longer survival. Furthermore, an optimal soil moisture content favors virus survival in soil, whereas desiccation results in a more rapid loss of viruses. Also remember that viruses (as obligate intracellular parasites) do not replicate outside of an appropriate host. Thus data characterizing their survival in sludge-amended soil are perhaps more straightforward.

Viral T_{90} values have ranged from less than 1 day under hot summer conditions to 56 days in the winter. Note that data presented in Table 2 that appear to support extended viral survival with soil depth actually reflect sampling to 20 cm in one Danish study where an average temperature of 0.5°C was observed. Undoubtedly, viruses can persist for extended periods in the soil environment where cold temperatures favor their survival. Studies completed under winter conditions in both Denmark and Ohio showed very similar inactivation rates, with T_{90} values of 30 days for two different enteric viruses. As evidenced by available data, however, inactivation at the soil surface can be quite rapid when viruses are exposed to high temperatures and drying conditions such as those prevailing in the southern United States during the summer and fall.

Among the parasites, protozoa seem to be very sensitive to drying, and under these conditions, survival rates are usually short. However, ova of helminths such as *Ascaris* are quite resistant to environmental stress. Parasites are also unable to replicate outside of their appropriate animal or human hosts.

Only three independent research groups have reported quantitative data for parasite survival in sludge-amended soil that allows estimation of inactivation rates. All but one T_{90} value is based on the addition of exogenus *Ascaris* or *Toxocara* ova to the sludge-soil system. As expected, T_{90} values for these parasitic forms exceeded those of all other microbial groups, ranging from 17 to 270 days, with a median value of 77 days (Table 2). Seasonal effects on ova survival were observed. Following summer sludge application, *Ascaris* ova were inactivated with apparent T_{90} values of 17 days in one study. Survival after applying sludge in the fall at the same location was more extended, with a T_{90} value of 65 days for *Ascaris* ova and 77 days for *Toxocara* ova. From another report, a T_{90} value of 30 days was estimated for *Ascaris* in sludge sprayed onto an untilled plot in the summer, whereas 90% inactivation following winter application required 80 and 90 days in separate experimental plots. Notably, after winter sludge application in this study, *Ascaris* ova survived dramatically longer, with a T_{90} of 200 days in tilled plots planted with a cover crop in the spring. Presumably, this extended survival time was favored by decreased soil temperature resulting from crop shading and/or by higher soil moisture resulting from irrigation and rainfall.

Attempts were made to analyze statistically the available quantitative data. Unfortunately, sufficient data were available only for temperature and die-off. Least-squares regression analyses of raw and transformed data were performed for the die-off recorded for salmonellae, fecal streptococci, fecal coliforms, total coliforms, viruses, and parasites. Only for fecal coliforms were there sufficient data to discriminate between soil depths. In the cases of salmonellae, viruses, and parasites, die-off at all depths was used in the analysis. Die-off at 5 to 15 cm was used in the analysis of salmonellae, fecal streptococci, and total coliforms.

Poor correlation was observed between organism inactivation and temperature for salmonellae, fecal streptococci, total coliforms, and parasites, whereas very good correlation was observed for fecal coliforms at both depths and for viruses. Results of this analysis for viruses appear in Figure 1 and illustrate some of the limitations of the available data. More often than not, the transformed T_{90}/T_{99} data correlated better with temperature, but the difference was not judged to be significant. Close scrutiny of these data shows that usable information for microorganisms such as viruses was available only at temperature extremes. Or, in other words, data tended to be clumped at either the warm or cold ranges, with few data in between. This observation restricts the value of an analysis over a range of temperatures and suggests the need for more detailed evaluation.

One approach to this evaluation was to use nonparametric correlations that make no assumptions about the normality of the distribution of the variables. The Kendall rank-order correlation was chosen for this evaluation. Only fecal coliform data at both the 0- to 5-cm depth and 5- to 15-cm depth were judged to be significant at the 5% level.

Transport of Microorganisms from Sludge-Amended Soils

In addition to survival of pathogens in sludge-amended soils, consideration must be given to their ability to move in this environment, either into surface waters through runoff or, perhaps more important, into groundwater through the soil profile. Though runoff may be viewed as largely the physical transport

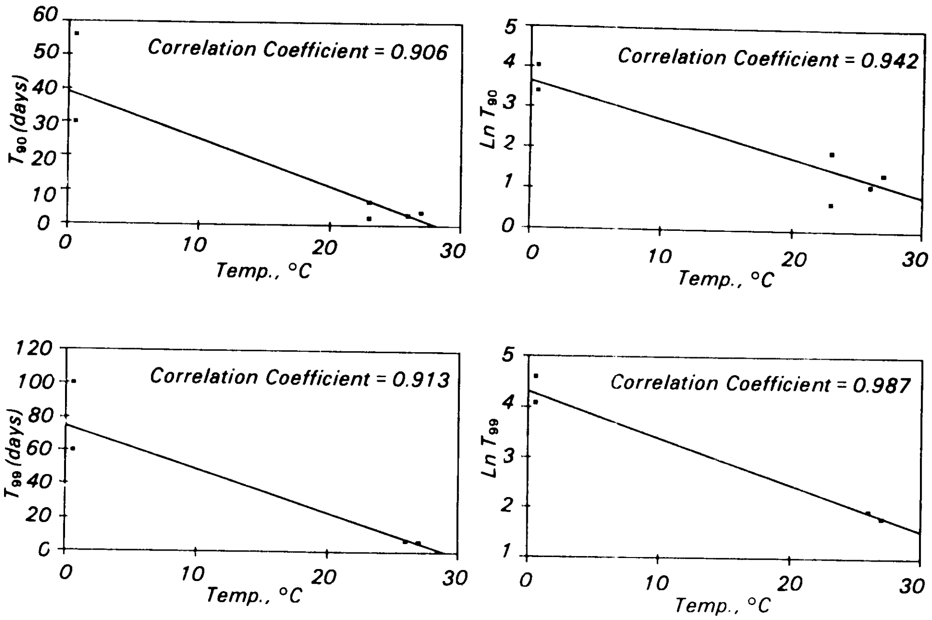

Figure 1. *Least squares regression plots for temperature and virus survival at all depths.*

of microorganisms associated with particulate material, vertical microbial transport is more complex.

Exceedingly few studies have addressed the presence of microorganisms in runoff from sludge-amended soils. No significant bacterial or viral impact has been observed on surface water at actual sludge application sites. However, as long as viable bacteria were present in sludge-amended soil, they were recovered at elevated levels from runoff intercepted at the lower end of sludge-amended test fields. Similarly, parasitic ova have been recovered from irrigation return flow at a sludge application site. Obviously, sludge application methods that minimize the displacement of sludge in surface runoff should be used if microorganism transport in runoff is to be avoided.

Removal of bacteria from wastewater percolating through a soil is due to both mechanical action (i.e., straining or sieving at the soil surface) and adsorption to soil particles. Similarly, the phenomenon of adsorption as a mechanism for retaining viruses in soil systems has been demonstrated. Release and movement of these microorganisms would be expected, since physical adsorption to particulates is a reversible phenomenon and, in part, ion-dependent.

The transport of protozoa and helminths in soils appears to be more limited than for bacteria or viruses. This may be the result of the considerable size differences between viruses, bacteria, and parasites. For example, protozoa are up to 20 times larger than bacteria and up to 2,000 times larger than enteroviruses. *Ascaris* eggs are even larger. Clearly, mechanical straining may be the most important factor governing the transport of these parasites.

Relatively few studies conducted at sludge application sites have looked for vertical transport of microorganisms. Limited monitoring has shown no demonstrable impact of sludge application on fecal coliform levels in groundwater. However, coliform bacteria have been detected sporadically at shallow depths of 100 and 180 cm beneath sludge-amended sites in the northwestern United States. In contrast, viruses have been recovered from relatively deep wells (8.5 and 18 m) at one sludge disposal site in Florida, whereas the results of groundwater monitoring at a second location in Florida were negative for viruses. Comparison of such results at operational field sites is often impeded, however, by the fragmentary nature of available data. Few studies have conducted integrated, long-term monitoring for viruses in which sludge, sludge-amended soil, and groundwater sampling were coordinated. Available literature strongly favors the contention that parasitic ova are retained at the point of sludge introduction. Groundwater monitoring for parasites has not been conducted and seems unnecessary given the relative size of most parasitic forms and their observed retention in the upper soil profiles.

Notwithstanding these limited field data, laboratory studies have demonstrated some transport of bacteria and viruses through sludge-amended soils. A single study has demonstrated movement of *Yersinia*, fecal coliforms, and fecal streptococci through 46 cm of soil, although maximal levels represented less than 0.1% of the bacteria present in the sludge. Note that experimental conditions in this study were chosen to represent a worst-case situation in which a total of 46 cm of rainfall was applied over a 3-week period.

Several laboratories have studied the vertical movement of viruses in model soil columns or cores. Studies in which lysimeters or cores amended with virally contaminated sludge have been exposed to natural rainfall have confirmed results obtained by groundwater monitoring at most sludge application sites. Specifically, in two separate studies, very few viruses were detected in soil water percolates intercepted at depths of 54 and 125 cm. Under certain laboratory test conditions, however, viruses applied with sludges have been transported through soil depths ranging from 13 cm in one study to 46 cm in a second study. When compared with the movement of free viruses, sludge-bound virions are much more effectively retained, apparently within the sludge-soil matrix at the point of application.

Conclusions

1. The number of quantitative, comparable data describing pathogen survival or transport in sludge-amended soils are extremely small. Survival data are available only for *Salmonella* sp., selected enteric viruses, and *Ascaris* ova, and studies on pathogen transport are limited to *Yersinia* sp. and certain viruses.
2. Where adequate quantitative data exist, these observations can be made:
   - Inactivation of indicator bacteria as described by median T_{90} values was greater than that observed for *Salmonella.*
   - Viral inactivation appears to be faster than *Salmonella* inactivation near the soil surface. However, all but one study used to estimate viral die-off were conducted in rather narrow temperature ranges, thus

highlighting a potential bias in the application of these values.

- Inactivation of parasites near the soil surface is relatively slow, perhaps as much as 5 times slower than *Salmonella* inactivation and more than 13 times slower than virus inactivation.

3. Exceedingly long survival times somtimes cited for *Salmonella* arise from studies in which high levels of added organisms (10^6 - 10^{10}/L) were present.
4. The only strong evidence for bacterial regrowth in sludge-amended soils is related to organisms of the coliform indicator group.
5. Of the physical and meteorological parameters considered, only temperature could be correlated with microorganism survival.
6. Inadequate data exist to assess critically the vertical transport of pathogens from sludge-amended soils. However, several general observations can be made:
   - Data collected at all but one operational field site have not demonstrated a deterioration of groundwater quality related to sludge application.
   - Selected studies have documented limited bacterial movement to depths of 180 cm beneath sludge-amended soil.
   - Limited laboratory studies suggest that viral retention is enhanced in sludge-amended soils compared with effluent-irrigated soils.
   - The size of parasites appears to preclude their vertical movement from sludge-amended soils, but studies designed to address this question were not found.
7. Exceedingly few studies have addressed the issue of microorganisms in runoff from sludge-amended soils. However, there is a high probability that uncontrolled runoff wil contain pathogens as long as viable organisms are present in sludge-amended soils.
8. Insufficient data are available for adequate modelling of pathogen survival or transport in sludge-amended soils. Not only are microbial results limited, but prevailing environmental and soil conditions have not been adequately documented in many published reports.

Recommendations

The following specific recommendations are designed to obtain the data required to formulate a more complete understanding of the survival and/or transport of pathogens in sludge-amended soils:

1. Studies specifically designed to develop such comprehensive data should be conducted.
2. These studies should be restricted to representative pathogens such as *Salmonella*, selected human enteric viruses, and parasites indigenous to municipal wastewater sludges.
3. Though it would be desirable to conduct such studies under field conditions with indigenous organisms, this approach may be limited by the levels of pathogens in sludges coupled with the relative insensitivity of currently used detection methods and the existence of a wide variety of uncontrolled environmental variables.
4. The use of selected seeded organisms in sludges under closely controlled laboratory conditions may be the most reasonable approach.
5. Laboratory experimentation must be carefully designed to simulate a range of temperature, moisture, sludge loading conditions, and soil types found nationwide.

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The complete report entitled "Survival and Transport of Pathogens in Sludge-Amended Soil: A Critical Literature Review." (Order No. PB 87-180 337/AS; Cost: $18.95, subject to change) will be available only from:
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