Hazards from Pathogenic Microorganisms in Land-Disposed Sewage Sludge

Timothy M. Straub,*† Ian L. Pepper,* and Charles P. Gerba*

Contents

I. Introduction

Municipal sewage sludge is a complex mixture of organic and inorganic compounds of biological and mineral origin that are removed from waste-

*Department of Soil and Water Science, The University of Arizona, Tucson, AZ 85721, U.S.A.
†Corresponding author.

Reviews of Environmental Contamination and Toxicology, Vol. 132.

water and sewage in sewage treatment plants. Sludge is a by-product of physical (primary treatment), biological (activated sludge, trickling filters, or rotating biological contractors), and physiochemical (precipitation with lime, ferric chloride, or alum) treatment of wastewater. Many of the pathogenic microorganisms present in raw wastewaters will find their way into municipal sludges. Treatment of these sludges by anaerobic or aerobic digestion and/or dewatering will reduce the number of pathogens, but significant numbers will remain. The type of treatment will determine the concentrations and relative risk of disposal.

Most work concerning the detection and implications of pathogens in sludge, or on soils amended with this product of sewage, were performed in the late 1970s and during the 1980s. This work concentrated on the inactivation of potential pathogens in sludge by various treatment processes used to chemically stabilize and reduce odor from the product. However, limited research has evaluated the fate of these potential pathogens after disposal on land or in large bodies of water until the late 1980s and early 1990s. This was due mostly to relatively insensitive techniques and/or the poor recovery of these pathogens from the soil and water environment.

Research into this topic was once again stimulated when the U. S. Environmental Protection Agency (USEPA) issued proposed standards for the removal of pathogens during the sludge treatment process in 1989 (USEPA 1989). Sewage treatment operators became concerned because many municipalities experienced dramatic growth during the period between the late 1970s to early 1990s. This led to significant increases in the volume of sewage handled by the existing facilities, with little to no expansion of the infrastructure to keep pace with demand. Although strict standards for effluent treatment and monitoring were maintained, many facilities were not equipped with adequate laboratories and trained personnel who could detect and monitor all the different pathogens in sludge required by the proposed regulations. Additionally, the USEPA would ban the disposal of sewage sludge into any body of water, fresh or marine, leaving only land disposal or incineration as viable disposal options.

The purpose of this review is to (1) discuss the types of pathogens and their concentrations in sludge that are of concern in terms of the USEPA-proposed standards, (2) review the literature on methods of sludge treatment in terms of the efficacy of pathogen reduction by these processes, (3) present data on the fate of selected pathogen groups after land disposal of sludge, (4) discuss exposure pathways for the transmission of pathogens in sludge to man, (5) introduce and discuss the newest advances for detection of these pathogens in the environment, (6) discuss risk assessment models for pathogens in sludge after disposal on land, and (7) outline future research needs to understand the fate and potential impact on human health from the land disposal of sewage sludge.

II. Origin of Sewage Sludge

Sewage sludge is a complex mixture of bio-solids resulting from precipitation processes during the various phases of sewage treatment. Raw sewage entering the wastewater treatment facility is first passed through a grit chamber to remove large debris. Primary treatment of sewage is a physical process whereby suspended solids are allowed to settle. These solids are termed primary sludge, (Hurst 1988). Primary effluent is further treated in a biological process to reduce biochemical oxygen demand, potential pathogens, and odor. This process can be accomplished by trickling filter, activated sludge, or rotating biological contactors. During this treatment process, organic matter is converted to CO_2, H_2O, and microbial biomass. Excess microbial biomass becomes secondary sludge, which is usually removed by settling. In certain treatment facilities where advanced wastewater treatment is performed, alum [$Al_2(SO_4)_3 \cdot 18H_2O$], ferric chloride ($FeCl_3$), or lime [$Ca(OH)_2$] is added to the secondary effluent. This causes flocculation of chemical constituents such as phosphates. Solids produced from this process are termed tertiary sludge. Raw sewage sludge, then, is a complex mixture of primary, secondary, and tertiary sludges depending on the level of wastewater treatment performed. A diagram of wastewater treatment is shown in Fig. 1.

Before land disposal or land application, raw sewage sludge must be treated to stabilize the decomposition of the organic matter, gas production, and to reduce the concentration of pathogens. Anaerobic digestion is

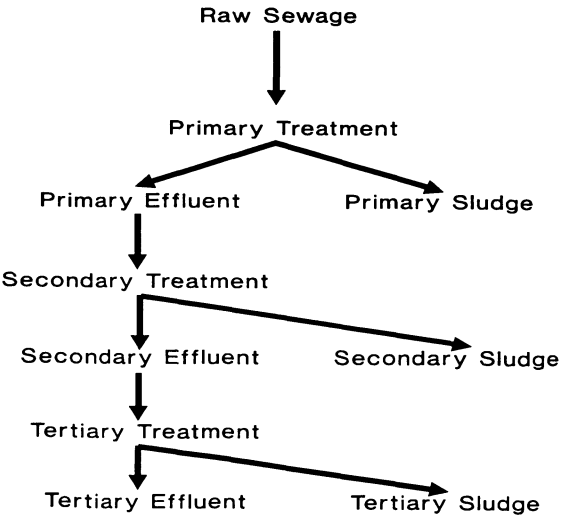

Fig. 1. Origin of sewage sludge. [Adapted from Hurst (1988).]

usually operated semicontinuously in large enclosed tanks called sludge digestors, into which untreated material is introduced and from which the treated material is removed at intervals. The retention time in the tank may range from 2 wk to 1 mon. The USEPA defines anaerobic digestion as those processes conducted in the absence of air at residence times ranging from 60 d at 20 °C to 15 d at 35–55 °C, with a volatile solids reduction of at least 38% (USEPA 1989). In aerobic digestion, sludge is stabilized by the passage of air through the sludge in a reactor. In a batch mode, the sludge is aerated for 2–3 wk.

III. Pathogens of Concern

Raw sewage may contain a wide variety of pathogenic microorganisms. The pathogens include bacteria, viruses, protozoa, helminths, and fungi, all of which can be expected to be present in raw, primary, and secondary sludges. Pathogens of concern are listed in Tables 1 and 2. It should be recognized that the list of pathogens is not constant. As advances in analytical techniques and changes in society have occurred, new pathogens are recognized and the significance of well-known ones changes. Microorganisms are subject to mutation and evolution, allowing for adaptation to changes in their environment. In addition, many pathogens are viable but nonculturable by current techniques (Rozak and Colwell 1987), and actual concentrations in sludge are probably underestimated. Thus, no assessment of the risks associated with the land application of sewage sludge can ever be considered to be complete when dealing with microorganisms. As new agents are discovered and a greater understanding of their ecology is developed, we must be willing to reevaluate previous assumptions.

A. Bacteria

Pathogenic bacteria can be found in large numbers in raw sewage sludge (Pepper and Gerba 1989). Such pathogens include *Salmonella typhi* (typhoid fever), *Shigella* spp. (Shigellosis), *Salmonella paratyphyi* (salmonellosis), and various species of *Escherichia coli* and *Campylobacter* sp. (gastroenteritis). The detection of individual pathogenic organisms is a difficult and time-consuming task. In practice, indicator organisms are used instead. Indicator organisms are those organisms associated with the intestinal tract whose occurrence indicates the potential presence of pathogenic bacteria. No one indicator organism satisfies all criteria necessary to predict the presence of all pathogens, hence, several indicator organisms are useful. Fecal coliforms are a subgroup of the coliform group and indicative of the feces of warm-blooded animals, and are usually lower in numbers than the total coliforms in sewage. Fecal streptococci are also a useful indicator organism, but they are usually less numerous than the coliform group in human feces.

Table 1. Human Viruses Shed in Feces That May Be Present in Sewage and Sludge.

Virus Group	Number of Serotypes	Illness Caused
Adenovirus	41	Pharyngitis, conjunctivitis, respiratory illness, vomiting, diarrhea
Astrovirus	5	Vomiting, diarrhea
Calicivirus	2	Vomiting, diarrhea
Coronavirus	1	Vomiting, diarrhea
Enterovirus		
Poliovirus	3	Paralysis, meningitis, fever
Coxsackie A	24	Herpangina, respiratory illness, meningitis, fever
Coxsackie B	6	Myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory illness, pleurodynia
Echovirus	34	Meningitis, encephalitis, respiratory disease, rash, diarrhea, fever
Enterovirus 68–72	4	Meningitis, encephalitis, respiratory illness, acute hemorrhagic conjunctivitis, fever
Hepatitis A virus	1	Hepatitis
Hepatitis E virus	1	Hepatitis
Norwalk virus	1	Epidemic vomiting and diarrhea
Reovirus	3	Not clearly established
Rotavirus	4	Vomiting, diarrhea
"Small round viruses"	2	Vomiting, diarrhea

Source: Modified from Hurst (1988).

Members of the genus *Salmonella* are the most widely recognized enteric pathogens. Often associated with food and waterborne outbreaks, they are responsible annually for 1–2 million human disease cases in the U.S. (Aserkoff et al. 1970). There are 2000 identified serotypes, many of which are able to infect both humans and animals. *Salmonella* has been studied more than any other pathogenic bacterium found in sewage, and a good deal is known about their removal during sewage treatment and survival in the environment. *Shigella* spp. are responsible for approximately 3% of the reported diarrhea cases in the U.S. (APHA 1975). The incidence of *Shigella* in a community is clearly related to sanitation and water quality (Feachem et al. 1983). Four groups of *Shigella* are recognized, but few data are available on their presence in the domestic wastes and survival in the environment because of the lack of good analytical methods for their detection.

Table 2. Bacteria and Parasites Pathogenic to Humans That May Be Present in Sewage and Sludge.

Group	Pathogen	Disease Caused
Bacteria	*Salmonella* (2000 types)	Typhoid, paratyphoid, salmonellosis
	Shigella (4 spp.)	Bacillary dysentery
	Enteropathogenic *E. coli*	Gastroenteritis
	Yersinia enterocolitica	Gastroenteritis
	Campylobacter jejuni	Gastroenteritis
	Vibrio cholerae	Cholera
	Leptospira	Weil's disease
Protozoa	*Entamoeba histolytica*	Amebic dysentery, liver abscess, colonic ulceration
	Giardia lamblia	Diarrhea, malabsorption
	Balantidium coli	Mild diarrhea, colonic ulceration
	Cryptosporidium	Diarrhea
Helminths	*Ascaris lumbricoides* (roundworm)	Ascariasis
	Ancyclostoma duodenale (hookworm)	Anemia
	Necator americanus (hookworm)	Anemia
	Taenia saginata (tapeworm)	Taeniasis (tapeworms from uncooked beef and pork)
	Trichuris (whipworm)	Abdominal pain, diarrhea
	Toxocara (roundworm)	Fever, abdominal pain
	Strongyloides (threadworm)	Abdominal pain, nausea, diarrhea

Source: Gerba (1983).

There are no data available on *Shigella* destruction in most sludge treatment processes (Feachem et al. 1983). However, it is believed that *Shigella* destruction will proceed more rapidly than for *Salmonella* or fecal indicator bacteria (Feachem et al. 1983).

Campylobacter spp. are now recognized as a significant cause of enteric illness in animals and man. The species of most concern as an enteric pathogen in humans is *Campylobacter jejuni*, now thought to be more prevalent than *Salmonella* and *Shigella* (Archer and Kvenberg 1985). Outbreaks have been linked to fecally contaminated food and water. Information on the occurrence of *Campylobacter* in sewage is limited due to problems associated with detection methodologies. In one study, the median concentration of *Campylobacter* spp. in sewage was determined to be $3.7 \times 10^3/100$ mL (Holler 1988). Jones et al. (1990a, 1990b) found that numbers of *Campylobacter* spp. in sewage sludge were dependent on season

and the length of time from sludge formation. Seasonal peaks occurred in May and June and appeared to be correlated with endemic infection in the community. It also appears that *Campylobacter* does not survive well in sewage sludge. In the same study performed by Jones et al. (1990b), no *Campylobacter* spp. were detected 2 d after sludge formation by primary sedimentation. The authors suggested that the organisms had reverted to a viable but noncultural state.

Vibrio cholerae causes cholera, an acute enteritis characterized by the sudden onset of symptoms and rapid dehydration. The study of *V. cholerae*, atypical *V. cholerae*, and non-O1 *V. cholerae* has been attracting increasing attention in recent years because of several seafood-associated *Vibrio cholerae* outbreaks along the Gulf Coast of the U.S. (Morris et al. 1981) and its recent widespread occurrence in Central and South America. It appears that *V. cholerae* may survive for prolonged periods in wastewater, especially at low temperatures (Feachem et al. 1983). In their review of the literature, Feachem et al. (1983) were unable to find any reports on the occurrence of *V. cholerae* in sludge or during sludge treatment.

It is only in the last few years that *Yersinia enterocolitica* has been recognized as an etiological agent of acute enteritis. Yersiniosis occurs only sporadically in the U.S. and is transmitted from either infected animals or humans. Food and waterborne outbreaks have been documented (Feachem et al. 1983), and the organism has been isolated from raw, digested, and dewatered sludges (Metro 1983).

Leptospira spp. are bacteria excreted in the urine of domestic and wild animals and enter municipal wastewater primarily from the urine of infected rats inhabiting sewers (Kowal 1985). Leptospirosis is uncommon in the U.S. (Kowal 1985) and survival is only 2–4 d in the environment (Feachem et al. 1983). The organism is rapidly destroyed during anaerobic sludge treatment, and survival is probably less than 2 d (Feachem et al. 1983).

Although *Escherichia coli* is usually considered nonpathogenic, enterotoxigenic and enteropathogenic variants are responsible for numerous outbreaks of enteritis. Several studies in different parts of the world have indicated that *E. coli* is a significant cause of bacterial diarrhea, and food and waterborne outbreaks have been documented (Feachem et al. 1983; Geldreich et al. 1992).

B. Viruses

Over 120 different viruses are excreted in human feces and urine and find their way into sewage. A listing of some of the viruses that could be found in domestic sewage and the diseases they may cause is provided in Table 1. Enteric viruses are those that can replicate in the gastrointestinal tract and be disseminated by the feces. They are divided into several groups based on morphological, physical, chemical, and antigenic differences. An infected individual may excrete as many as 10^{10} viruses per gram of feces and will

continue to shed them into the sewage stream for up to 50 d (Melnick and Gerba 1980).

Raw sewage entering the wastewater treatment plant contains significant numbers of viral pathogens. Estimated enteric virus densities may be in excess of 7000/L of raw sewage in the U.S. (Melnick et al. 1978). Unfortunately, viruses have been shown to concentrate in sludge (Ward and Ashley 1977; Wellings et al. 1976). Furthermore, the treatment of raw sewage sludge may not effectively reduce the number of infectious viruses. Soares (1990) found that viral reduction in anaerobically digested sewage sludge ranged from less than 50% to greater than 99.9%, with a high degree of variability in treatment efficiency. In addition, the concentration of viruses leaving the digester could be in excess of 1000 viruses/L even if treatment efficiency were 99%. In Florida, Wellings et al. (1976) reported concentrations of 24 plaque-forming units (pfu) of virus /250 g of sludge cake. The viruses in this study were identified as echovirus 7, which is known to cause encephalitis in man.

Most of the knowledge on viruses in sewage is in regard to those associated with gastroenteritis. Exceptions are certain enteroviruses that are associated with a wide variety of diseases and adenoviruses, which may cause eye and upper respiratory infections. Enteroviruses are often associated with more serious illnesses such as hepatitis, meningitis, myocarditis, and paralysis (Table 1).

The most commonly studied enteric viruses in sewage and sludge are the enteroviruses that include the polioviruses, coxsackie A and B viruses, echoviruses, and other recently classified enterovirus types. Although many of the enterovirus infections, such as those caused by poliovirus, may be asymptomatic, symptomatic infections may be as high as 95% during outbreaks of hepatitis (Lednar et al. 1985). A great deal of information is available on the removal of enteroviruses by sewage treatment, and many studies have been conducted on their occurrence in sludge (Leong 1983).

Rotaviruses are now recognized as a major cause of childhood gastroenteritis, sometimes resulting in dehydration and death in infants and adults (Gerba et al. 1985). Several waterborne outbreaks have been documented (Gerba et al. 1985; Williams and Akin 1986) and the virus isolated from sewage sludges (Gerba 1986).

The Norwalk virus has been demonstrated to be the cause of numerous waterborne outbreaks of epidemic gastroenteritis (Gerba et al. 1984). Since methods have not been developed for its isolation in cell culture, its occurrence and concentration in sewage sludge are unknown. Astroviruses, caliciviruses, coronaviruses, and several other Norwalk-like agents have been associated with human gastroenteritis, but little is known about them. Laboratory methods are currently not available to study most of these agents, and they await further characterization.

Adenoviruses primarily cause respiratory infections and eye infections, although several new types have been found associated primarily with gastroenteritis (Gary et al. 1979).

Hepatitis E virus has recently been recognized as a cause of waterborne disease outbreaks in Asia and Africa and has recently been grown in cell culture (Huang et al. 1992). It appears to be related to the Calciviridae family.

C. Protozoa

In the past, little attention had been given to the presence of parasites in sewage because of the popular impression that the prevalence of parasite infection in the U.S. is low (Larkin et al. 1976). However, the continuing occurrence of waterborne outbreaks of giardiasis and the resistance of cysts to disinfection indicate that they deserve serious consideration (Erlandsen and Meyer 1984).

Of the common protozoa that may be found in sewage, only four species are believed to be of major significance for the transmission of disease to humans: *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, and *Cryptosporidium* spp. All four cause mild to severe diarrhea. Waterborne outbreaks for all of these agents have occurred. *G. lamblia* is now the agent most commonly associated with waterborne outbreaks in which an agent has been identified in the U.S. (Herwaldt et al. 1991, 1992). *Cryptosporidium* spp. have only been recognized as pathogens in humans. They infect both animals and people and are apparently a cause of travelers' diarrhea and gastroenteritis worldwide (Smith and Rose 1990). A waterborne outbreak of cryptosporidiasis in the U.S. was first documented in Texas in 1985 (D'Antonio et al. 1985). Additional outbreaks have since occurred in the United Kingdom (Smith and Rose 1990). In the U.S., *Cryptosporidium* has been identified from domestic sewage effluents (Musial 1985) and sludge (Kayed 1986)

On a per-kilogram basis, *Giardia* cysts usually ranged from 10^4–10^6 in the treated sludge. The average concentration of *Giardia* cysts was 1.46×10^4/L in raw sludge and 1.47×10^4/L in treated sludge (Soares 1990). No removal or destruction of *Giardia* cysts occurred during treatment, although the viability of the cysts may have been significantly reduced. Average concentrations of cysts were 6.23×10^5/kg or 623/g. According to USEPA guidelines, class A treated sludge should contain less than one protozoan cyst/g. Although high levels of cysts have been detected, no excystation procedures were performed to determine cyst viability and the health risks of *Giardia* infection after sludge treatment. Recently, Gavaghan et al. (1993) assessed the inactivation of *Giardia muris* cysts in a laboratory anaerobic digestor. The results showed that 99.9% of the cysts were inactivated (failed to excyst) within an 18 hr exposure to 37 °C.

D. Helminths

A wide variety of helminths and their eggs may occur in domestic sludges (Gerba and Bitton 1984). Helminths are worms that include nematodes (roundworms) and cestodes (tapeworms). Those of primary concern are

listed Table 2. Many common helminths are pathogenic to domestic animals (e.g., cats and dogs) and sometimes identified in domestic wastewater and sludge, but are not pathogenic to man. Reimers et al. (1981) have found *Ascaris*, *Trichuris*, and *Toxocara* helminth eggs in municipal wastewater sludge in both the southeastern and northern U.S..

Ascariasis is a helminthic infection of the small intestine by the human roundworm *Ascaris lumbricoides*. About 85% of the infections are asymptomatic, although the presence of a few worm eggs is potentially infectious (Feachem et al. 1983). Large numbers of worms may cause digestive and nutritional disturbances, abdominal pain, and damage to internal organs. The prevalence of ascariasis in the U.S. was estimated at about 4 million in 1972 (Warren 1974).

Ascaris eggs tend to become concentrated in the sludge during sewage treatment and their removal by sludge treatment has been studied (Feachem et al. 1983).

Trichuriasis is an infection of man by the human whipworm, *Trichuris trichiura*. Trichuriasis is a helminthic infection of the large intestine and cecum. Most infections in adults are asymptomatic, but there may be slight abdominal pain and diarrhea. *Trichuris* eggs, like *Ascaris* eggs, tend to settle in primary and secondary sedimentation tanks and, therefore, are concentrated in the sludge from sewage treatment plants. The fate of *Trichuris* eggs during storage, digestion, or composting is believed to be the same as that for *Ascaris* eggs (Feachem et al. 1983).

Ancyclostomiasis is an infection of the small intestine with one of the two species of human hookworms: *Necator americanus* or *Ancyclostoma duodenale*. Ancyclostomiasis is frequently symptomless. When it does produce illness it constitutes a public health problem. The most important features are anemia and debility. Because of the low incidence of hookworm in the U.S., only low numbers have been found in sludge. Hookworm eggs and larvae are less resistant to the sludge treatment process than *Ascaris* eggs (Feachem et al. 1983). Problems could arise if raw or inadequately treated sludges are applied to pastureland, since once in the soil, the eggs will hatch, thereby producing infective larvae.

Taenia saginata and *T. solium*, the beef and pork tapeworms, live in the intestinal tract where they may cause abdominal pain, weight loss, and digestive disturbances. The infection arises from eating incompletely cooked meat containing the larval stage of the tapeworm, rather than from wastewater-contaminated material. Man serves as the definitive host, harboring the adult. The eggs are passed in the feces, ingested by cattle and pigs (intermediate hosts), hatch, and the larvae migrate into the tissues, where they develop into the cysticercus stage. The hazard is then principally to livestock grazing on land application sites. *Taenia* eggs are concentrated in sewage sludge and may survive for prolonged periods after land disposal (Feachem et al. 1983). *Taenia* eggs may not be completely destroyed by all sludge treatment processes (Feachem et al. 1983). An investigation of a *T.*

saginata outbreak near Tucson, Arizona, revealed that cattle became infected while grazing on a pasture irrigated with primary sewage effluent (Slonka et al. 1975). Pastureland fertilized with municipal sludge was implicated in a *T. saginata* outbreak in Virginia (Hammerberg et al. 1978).

E. Fungi

Fungi are usually considered to be of minimal health risk in the application of municipal sludge. The pathogenic fungi listed in Table 3 can all be recovered from municipal sludge (WHO 1981). These fungi can form two groups: the yeasts and filamentous molds. The yeasts include *Candida albicans* and other *Candida* spp., *Cryptococcus neoformans*, and *Trichosporon* spp., whereas the filamentous mold varieties include the various species of *Aspergillus*, especially *A. fumigatus*, *Epidermophyton* spp., *Phialophora* spp., and *Trichlophyton* spp. These fungi have been reported in sewage and in all stages of sludge treatment (WHO 1981). *Aspergillus fumigatus* is one of the most prevalent fungi in municipal compost. This opportunistic pathogen may cause upper respiratory tract infections in man (WHO 1981). Because fungi are environmentally ubiquitous, it is difficult to evaluate their significance to public health. The World Health Organization's Working Group on Sewage Sludge to Land:Health Implications of the Microbial Content (WHO 1981) emphasized that because of their presence in nature, even if the sludge was treated by pasteurization, recontamination of the sludge will occur.

IV. Incidence of Pathogens in Sludge

Concentrations and types of pathogens in sludges depend on two principal factors: the incidence of infection within a community and the type of sludge treatment. Season, climate, and sanitation are major factors determining the pathogen load that a wastewater treatment plant will receive.

Table 3. Fungi Pathogenic to Man That May Be Present in Sewage and Sludge.

Pathogen	Diseases Caused
Aspergillus fumigatus	Respiratory otomycosis
Candida albicans	Candidiasis
Cryptococcus neoformans	Subacute chronic meningitis
Epidermophyton spp. and *Trichophyton* spp.	Ringworm and athlete's foot
Trichosporon spp.	Infection of hair follicles
Phialophora spp.	Deep tissue infections

Source: Gerba (1983).

Various sludge treatment processes, such as anaerobic digestion and dewatering, will act to reduce the numbers of some pathogens initially present.

A. Pathogen Concentrations in Primary Sludge

Most microbial species contained in raw sewage are concentrated in sludge during primary sedimentation. Enteric viruses have too little mass to settle alone, but because of their strong binding affinity to particulates, they also are concentrated in sludge (Ward and Ashley 1977).

Densities of microorganisms shown in Table 4 represent typical, average values detected by various investigators. Different sludges may contain significantly greater or less numbers of any organism as determined primarily by the kind of sewage from which the sludge was derived. The quantities of pathogenic species will be especially variable depending on which are present in a community at any particular time. Indicator organisms are normally present in fairly constant amounts. Because concentrations determined in any study are dependent on the assays for each microbial species, these concentrations are only as accurate as the assays, due to inefficient recovery of viruses from environmental samples.

B. Pathogen Concentrations In Secondary Sludge

The secondary sludges of concern in this report are produced following the biological treatment of wastewater. Microbial populations in sludges following these treatments depend on the initial concentrations in the wastewater, die-off or growth during treatments, and the association of these

Table 4. Densities of Microbial Pathogens and Indicators in Primary Sludges.

Type	Organism	Density (#/g dry wt)
Virus	Various enteric viruses	10^2–10^4
	Bacteriophages	10^5
Bacteria	Total coliforms	10^8–10^9
	Fecal coliforms	10^7–10^8
	Fecal streptococci	10^6–10^7
	Salmonella sp.	10^2–10^3
	Clostridia sp.	10^6
	Mycobacterium tuberculosis	10^6
Protozoa	*Giardia* sp.	10^2–10^3
Helminths	*Ascaris* sp.	10^2–10^3
	Trichuris vulpis	10^2
	Toxocara sp.	10^1–10^2

Source: Modified from Ward et al. (1984).

organisms with sludge (Ward et al. 1984). Some treatment processes such as the activated sludge process have a deleterious effect on enteric microbial species. Viral and bacterial pathogens have been reduced in concentration by activated sludge treatment. Even so, the ranges of concentration in secondary sludges obtained from this and most other secondary treatments are usually not significantly different from those of primary sludges. Examples are shown in Table 5.

V. Methods of Sewage Sludge Treatment and Their Efficacy in Pathogen Removal

Sludges resulting from the treatment of domestic sewage need to be treated (1) to reduce organic matter and water content, (2) to remove unpleasant odors from the incomplete oxidation of organic matter, and (3) for the purposes of this discussion, to reduce the concentration of pathogens to proposed USEPA regulations. There are four basic methods of sludge treatment, each with its own unique advantages and disadvantages. These treatment processes include mesophilic or thermophilic anaerobic digestion, aerobic sludge digestion, composting, and lime stabilization. In light of the new regulations, treatment facilities may use a combination of these methods to achieve the desired pathogen reduction.

Anaerobic digestion can be mesophilic (temperature from 30–38 °C) or thermophilic (50–60 °C) (Pederson 1983). High-rate reactors are commonly used to mix the sludge under anaerobic conditions, and the reaction is heated to either mesophilic or thermophilic conditions. Low-rate reactors, which are more typical of a septic tank system, allow the sludge to settle,

Table 5. Densities of Pathogenic and Indicator Microbial Species in Secondary Sludges.

Type	Organism	Density (#/g dry wt)
Virus	Various eneteric viruses	3×10^2
Bacteria	Total coliforms	7×10^8
	Fecal coliforms	8×10^6
	Fecal streptococci	2×10^2
	Salmonella sp.	9×10^2
Protozoa	*Giardia* sp.	10^2–10^3
Helminths	*Ascaris* sp.	1×10^3
	Trichuris vulpis	$< 10^2$
	Toxocara sp.	3×10^2

Source: Modified from Ward et al. (1984).

and reactions proceed anaerobically for 30–60 d. Larger municipalities use anaerobic digestion to treat sludge, because methane gas produced during the process can be recovered and used to supply some of the energy needs of the facility (Bitton 1980). It has the additional advantage of not requiring an input of air or oxygen into the system, which is a costly feature in treatment facilities using aerobic digestion to treat sludge (Pederson 1983).

The reduction of pathogenic microorganisms by anaerobic digestion is both time- and temperature-dependent. Thermophilic digestion and longer detention times favor greater reduction of potential pathogens (Ward and Ashley 1977). In general, a plant using mesophilic digestion with a mean retention time of 14–15 d can expect 1–2 $\log_{10}$ removal of total coliforms, fecal coliforms, and fecal streptococcus (Berg and Berman 1980; Jewell et al. 1980; Lue-Hing et al. 1977; Pederson 1983). Helminth ova apparently survive anaerobic digestion with little reduction in viable numbers (USEPA 1986).

Smaller treatment facilities may use aerobic digestion to treat sewage sludge (Bitton 1980). Temperatures for aerobic digestion are usually mesophilic (37 °C) with a mean retention time of 10–20 d. Air must be pumped into the reaction tanks, which increases costs due to the energy input. Pathogen reduction may also be limited (Pederson 1983). Conversion of organic matter into carbon dioxide and water leads to decreased carbon sources for bacteria; hence, the numbers of bacteria are most likely reduced due to nutrient deprivation. Less than a 1 $\log_{10}$ reduction of enterovirus was observed when aerobic digestion was used to treat sludge [(Bitton et al. 1984), cited by Pederson (1983)].

Mesophilic composting is another means of sludge treatment. Liquid sludge is mixed with a bulking agent such as wood chips, dry compost, or municipal refuse. Naturally occurring microorganisms within the pile can increase the temperature inside to 60 °C or greater (Pederson 1983). The temperature increase is due to oxidation of utilizable substrates present in sludge by microorganisms (Atlas and Bartha 1987). After nutrient sources are exhausted, the pile cools to ambient temperatures and the organic matter of the sludge has been mineralized to CO_2 and H_2O or transformed into humiclike substances similar to stable soil organic matter. There are three basic methods of composting.

In the windrow system, sludge is mixed with other materials and formed into long piles perhaps 2.25 m high, 3 m wide, and at least 6 m long. Piles are turned periodically to allow aeration. Total time can take 6–10 wk depending on climate and the specific composting mix. In the Beltsville system, developed in Beltsville, Maryland, air is blown into or sucked through the compost pile to achieve the desired aeration of the compost pile. This increased aeration shortens the composting period to 3–4 wk. The rotating drum method is a system in which the sludge compost is contained in a well-aerated rotating drum for 2–3 d. Temperatures in this system can exceed 70 °C. This system controls the environmental factors affecting the composting more than the previous two methods.

The main factor controlling the fate of pathogens would be temperature and time. Temperatures within the pile are extreme enough to inactivate enteric viruses 3–4 $\log_{10}$ [(Cramer and Burge 1975; Ward and Ashley 1978); cited by Pederson (1983)], indicator bacteria 3–4 $\log_{10}$ [(Epstein et al. 1976; Lacoboni and LeBrun 1977); cited by Pederson (1983)], and possibly protozoan and helminth parasites (i.e., 3 $\log_{10}$ for *Ascaris lumbricoides* at temperatures of 50 °C for 1 hr) [Cramer and Burge 1975); cited by Pederson (1983)]. However, temperatures at the outer edges of the pile are not expected to be lethal to microorganisms, and the pile could become reinoculated by turning the pile. In fact, even at the center, where the temperatures are the most extreme, the number of viable and culturable mesophiles can be in excess of 10^8/g of compost (Atlas and Bartha 1987). The regrowth of bacterial pathogens such as *Salmonella* is also a possibility.

A fourth method for treating sludge is lime stabilization (Pederson 1983). In this process, liquid sewage sludge is mixed with a sufficient amount of lime to raise the pH to 12.0 for at least 2 hr. At this pH, the NH_4^+ ion is deprotonated, resulting in the production of ammonia gas. The combination of high pH and ammonia can reduce enteroviruses by four orders of magnitude (Sattar et al. 1976), coliform indicator bacteria two to seven orders of magnitude (Counts and Shuckrow 1974), but very little reduction of fecal streptococcus indicator bacteria exists (Counts and Shuckrow 1974), and no reduction of parasites (Remiers et al. 1980).

Other nonconventional treatment or disinfection processes such as heat drying, pasteurization, heat treatment, and γ-irradiation will also act to reduce the numbers of pathogens present in sludge before disposal. Their effectiveness on pathogen removal is discussed by Ward et al. (1984).

A summary of expected microbial reduction by the various sludge treatment processes is listed in Table 6. Expected concentrations after digestion

Table 6. Summary of Microbial Reduction During Sludge Treatment.

	Reduction[a]		
Treatment	Bacteria	Viruses	Parasites
Anaerobic digestion[b]	1-2	1	0
Aerobic digestion	1-2	1	0
Composting	2-3	2-3	2-3
Air drying[c]	2-3	1-3	1-3
Lime stabilization	2-3	3	0

Source: Modified from Ward et al. (1984).

[a]Scale: 0 = <0.5 orders of magnitude (< 10% reduction); 1 = 0.5–2 orders of magnitude (99% reduction); 2 = 2–4 orders of magnitude (99.9% reduction); 3 = > 4 orders of magnitude (99.99% reduction).

[b]Mesophilic temperatures (27–37 °C) assumed.

[c]Effects depend on moisture levels.

of sludge are given in Table 7. It is important to note that despite a 1–2 $\log_{10}$ decrease in bacterial and viral numbers, significant concentrations of these pathogens persist after sludge treatment (Pepper and Gerba 1989; Soares 1990). In the absence of excystation procedures, parasite concentrations are not reduced by sludge treatment.

VI. Land Disposal of Sewage Sludge

Amendment of sewage sludge to nonfood agricultural production lands is perhaps the most economical means of sewage sludge disposal. Disposal of liquid anaerobically digested sewage sludge (1–2% solids) benefits agriculture both by its fertilization and irrigation value (Straub et al. 1992). There are three methods in which liquid sludge is applied to land: (1) surface spreading by tankers, (2) surface spreading by rain gun, and (3) sludge injection.

Surface spreading by tankers is perhaps the most cost-effective method to dispose of sludge. One disadvantage of this method is the uneven spreading of sludge (Wallis and Lehman 1983). This is mostly due to problems associated with maintaining constant speed of the tanker and uneven topography of the field. As a result, the sludge tends to accumulate in pockets. The second disadvantage is that the amount of sludge that can be loaded on the field is regulated by soil compaction, which relates to the increase in bulk density of the soil from sludge application. Pepper et al. (1991) reported that sludge application increased the bulk density of the clay loam

Table 7. Pathogen Concentrations and Indicators in Digested Sludges.

	Type of Treatment (g dry wt)	
Organism	Anaerobic	Aerobic
Enteroviruses	0.2–210	0–260
Rotaviruses	14–485	ND
Salmonella	$3–10^3$	3
Total coliforms	$10^2–10^6$	$10^5–10^6$
Fecal coliforms	$10^2–10^6$	$10^5–10^6$
Shigella sp.	20	ND
Yersinia enterocolitica	10^5	ND
Giardia sp.	$10^2–10^3$	ND
Ascaris	–	–
Trichuris	–	–
Toxocara	–	–

Source: Modified from Ward et al. (1984).
ND = not determined.

and silty clay loam soils to the point where soil respiration and hence plant growth were actually reduced. Usually, the application is worked into the soil within the hour of sludge application (Wallis and Lehman 1983).

When sludge is applied by rain guns, solids need to be less than 1–2%. Irrigation sprayers are often modified to allow the sludge to pass through the sprayer. This method has problems similar to surface spreading. It may also lead to the aerosolization of pathogenic microorganisms. As with surface spreading, the sludge should be worked into the soil as soon as possible.

Subsurface sludge injection reduces the problem of uneven spreading characteristics of the previous two methods. Sludge injectors provide a continuous ribbon of sludge at preset depths and rates. It also has the advantage of reducing odor and animal vectors that may carry pathogens significant distances from the disposal site. Its major disadvantages are expense of the equipment and prolonging the survival of pathogenic microorganisms due to reduced desiccation, extremes of temperature, and ultraviolet rays from the sun (Wallis and Lehman 1983).

If the solids content of sludge is greater than 15% (dewatered stabilized and unstabilized), the sludge may be disposed of in a landfill. The kind of landfill depends on site characteristics and those of the sludge itself.

Sewage sludge stabilized by thermophilic composting with other organic materials such as wood chips, decaying plant material, or other solid waste converts the organic material within the pile to organic matter similar in structure to soil organic matter (Atlas and Bartha 1987). The finished compost can be sold as fertilizer on a small-scale basis (home-gardening projects) or pelleted for agricultural use.

VII. Exposure Pathways

The possible exposure pathways by which infectious microorganisms may come into contact with humans during the operation of sludge landfills or sludge amendment to agricultural soil are shown in Fig. 2. The consequence of exposure to one or more routes of transmission is dependent on the likelihood of a significant number of microorganisms being present in sludge that might result in infection. All of the pathogens present in sludge may follow the pathways illustrated in Fig. 2; however, it is unlikely that significant numbers are transmitted by all pathways.

Exposure of personnel may occur through direct contact with sludge or exposure to aerosols generated during burial. Aerosols could also be transported downwind to exposure areas distant from the disposal site. Aerosols containing viable microorganisms also represent a means of direct contamination of clothing and equipment. Microorganisms may leach from buried sludge with infiltrating water to contaminate groundwater. Exposure of the sludge to the surface would result in the generation of runoff, which may transport sludge particles to nearby surface waters. It is also possible

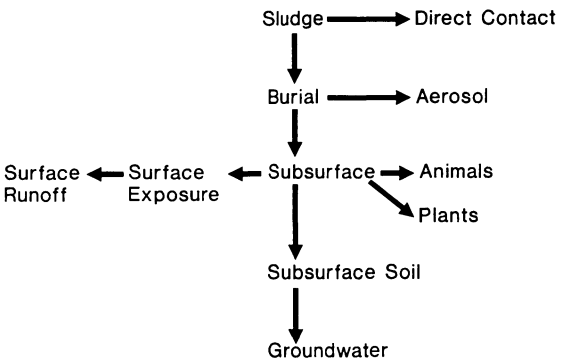

Fig. 2. Exposure pathways for pathogens from land disposal of sewage sludge.

that, if the site becomes saturated with water, surface leachate contamination will occur. Burrowing animals could come into contact with the buried or injected sludge and birds could be exposed to the sludge before burial. These animals could transport sludge material off site or expose it to the surface. The translocation of viruses from plant roots to aerial parts of the plant is another potential pathway.

Many enteric microorganisms can effectively be transmitted by aerosols. Aerosols of enteric organisms are generated during sewage treatment and the spraying of sewage effluents and sludges onto land (Pahren and Jakubowski 1980). Organisms in such aerosols can be transmitted by inhalation or human contact with contaminated surfaces.

The number of microorganisms in aerosols depends on the type of sludge disposed, method of application, and number of microorganisms in the sludge. The greatest amount of aerosol generation would occur during the application of sludges with a low solids content applied as slurries during spray application. Dumping of sludges from trucks onto the soil or into trenches and area fills would also generate aerosols on impact. Some aerosoling would occur during the injection of sludge. Greater numbers of pathogenic microorganisms would be aerosoled during disposal of primary rather than treated sludges.

If wind velocities at a site are great enough, suspension sludge particles could occur (USEPA 1986). Most sludges would not be easily resuspended because of their moisture content and tendency to mat as they dry. Dried sludges, however, may be very light and fine in texture and, therefore, easily resuspended. If dried sludge is not covered at windy sites, winds will attain speeds capable of suspending the sludge from the working face. However, even at the windiest sites, sludge resuspension will only occur for brief periods. Such events could be controlled by requiring the placement of a soil cover daily over landfilled sludges (USEPA 1986).

The possibility of health risks from public and occupational exposures to these aerosols has been discussed extensively (Pahren and Jakubowski 1980). Several studies have dealt with the measurement of aerosols from activated sludge treatment plants and spray irrigation of wastewater to land. These studies included aerosol monitoring and attempts to examine the health effects in populations either working at the site or living nearby. However, epidemiological studies have not produced conclusive results as to the impact of such aerosols on human health (Pahren and Jakubowski 1980).

The use of tank trucks and high-volume spray guns for the application of liquid sludges would be expected to have a much greater chance of generating significant microbial aerosols compared to landfilling or surface spreading. Sorber et al. (1984), in a study of aerosols generated during land application of liquid sludge, reported that microbial aerosol concentrations are less than those at wastewater spray application sites, and no significant health effects should occur for individuals located greater than 100 m downwind of application.

In summary, some aerosoling of pathogenic organisms will occur with some possible risk of disease transmission. Through proper management and the use of a buffer zone, significant microbial aerosols should not occur off site.

During sludge landfill operations, it is normal practice to bury the sludge under several feet of earth at the end of each day. Even in those operations, where the sludge may be exposed for several days, the sludge is contained in trenches or pits that limit exposure to surface runoff. However, surface spreading of treated sludge on non-food-producing agricultural lands often leaves pools of liquid sludge, and sudden rainfall or irrigation events, common in the desert Southwest, can contaminate recreational surface, irrigated food crops, and drinking water, possibly posing a threat to human health.

Potentially, plant roots and burrowing animals could come in contact with the buried or surface-spread sludge. In addition, birds could become exposed to sludge before its burial. The translocation of viruses from the roots to the aerial parts of plants has been observed (Murphy and Syverton 1958; Ward and Mahler 1982), but only when grown in hydroponic culture or when roots were cut. Ward and Mahler concluded that it was unlikely that viruses penetrate intact root surfaces. Contamination of groundwater used for domestic purposes appears the most likely route of significant human exposure from sludge applied to soil. Many of the sludge application sites in the U.S. are operated over aquifers used as potable sources and these sites are within a few meters of the groundwater table. Also, any field receiving liquid-treated sewage sludge several meters above an aquifer used as a potable water source could put users at risk. Therefore, this pathway is considered to be the most significant.

VIII. Survival of Pathogens in Soil and Sludge

A. Viruses

In a recent study, Straub et al. (1992) investigated the fate of three different viruses in two desert soils amended with anaerobically digested sludge. The viruses used were poliovirus type 1, a typical enteric virus routinely isolated from sewage, and two bacteriophages, MS2 and PRD-1, used extensively to model the fate of enteric viruses in the field. Soils used were a clay loam typical of the agricultural land where sludge is applied routinely and a sandy loam soil typical of land adjacent to dry washes. The investigators were interested in the survival of viruses at constant temperature and soil moisture and the combined effects of temperature and moisture loss.

When both temperature and soil moisture were maintained, temperature was the key factor in determining virus survival. In general as temperature increased, virus survival decreased. However, although this assumption generally holds true for all viruses in soil (Bagdasar'yan 1964; Yeager and O'Brien 1979), each kind of virus has quite different survival characteristics. MS2, used to model the fate of poliovirus based on its size and structural similarities (Powelson et al. 1991), was found to be 10 X less resistant to thermal inactivation than poliovirus. However, poliovirus was even less resistant to thermal inactivation than PRD-1. There was no observable inactivation of bacteriophage PRD-1 until the temperature was increased to 40 °C.

In this study, all three viruses survived longer in sludge-amended clay loam soils than in sludge-amended sandy loam soils. This was observed for MS2 in the 15° and 27 °C study, poliovirus in the 15° and 27 °C study, and PRD-1 in the 40 °C study. Gerba et al. (1981) and Hurst et al. (1980) reported similar observations and suggested that charges on the clay mineral stabilize the nucleic acid if adsorbed to the particle. This stabilization may prevent soil nucleases from attacking viral nucleic acids. Although viruses can remain viable longer in sludge-amended clay loam soils, saturated hydraulic conductivity could be much less, depending on soil structure, than sludge-amended sandy loam, leading to retention of the viruses at the surface due to restricted water movement through the soil.

Straub et al. (1992) further studied the combined effects of temperature and evaporation. The design was similar to constant moisture studies except that moisture was allowed to evaporate. When soil moisture decreased from 35% (approximately saturated conditions) to less than 5%, no virus was recovered after 7 d regardless of temperature. In some cases, viruses were more rapidly inactivated in drying sludge-amended clay loam soils than drying sludge-amended sandy loam soils even though their moisture, contents were similar. This was contrary to the belief that clay soils protect viruses from inactivation more than sandy soils. The authors explained that matrix potentials would be a better indication of water availability for biological activity, and that soils at identical water content have different

matrix potentials. A clay loam and sandy loam at 10% moisture may have matrix potentials of -30 and -10 bars, respectively. In the absence of matrix potential data, a previous study on virus survival in soil reported that the critical soil moisture content for a virus was approximately 2.9% (w/w) in a New Mexico coarse sandy loam (Yeager and O'Brien 1979). At soil moisture above 2.9%, virus survival was a function of temperature. Below 2.9%, evaporation was the factor governing inactivation. It was further shown by radioactive labeling that, at or below this moisture, conformational changes occurred in the protein capsid, causing the nucleic acid to be ejected. Naturally occurring nucleases in the soil quickly degraded the intact nucleic acid sequence, indicating that viruses were not irreversibly adsorbed to soil.

When virus survival in sludge-amended soil is assessed in the field, results will vary depending on climate, method of sludge disposal, and, if applied to agricultural land, irrigation practices. Cold and moist climates tend to favor increased viability, whereas hot and dry climates favor rapid inactivation of viruses (Bagdasar'yan 1964; Yeager and O'Brien 1979). Moisture can be maintained by frequent rainfall events and irrigation. Additionally, deep burial can lead to decreased evaporation. The surface spreading of sludge may be beneficial since maximum exposure to air leads to the greatest evaporation.

Sorber and Moore (1987) were able to recover viruses from a sludge burial site in Montana 6 mon after the last disposal in October. The two main factors for the prolonged virus survival were low winter temperatures and burial depth. Soil temperature in Montana from October through April is less than 5 °C. Additionally, burial of liquid sludge would impede evaporation. Free waterflow would equilibrate, but would likely not reach the critical moisture content suggested by Yeager and O'Brien (1979).

Bitton et al. (1984) studied survival of viruses in Florida during the hot and dry season. Sludge was injected 10 cm below the soil surface where the average soil temperature was 27 °C. These investigators were unable to recover viruses 8 d postsludge injection. The two factors of higher soil temperature and evaporation probably combined lead to the rapid inactivation. Here, evaporation would be possible at 10 cm, and it is often observed that liquid sludge injected at this depth can rise to the surface, especially if the hydraulic conductivity of these soils is moderately low.

Straub et al. (1993) investigated the survival of poliovirus and MS2 in the field during winter and summer in Arizona. During the winter months, no inactivation of poliovirus was observed after 10 d and less than a 2 $\log_{10}$ reduction of MS2 virus was observed. Frequent winter rains maintained soil moisture content at approximately field capacity. When repeated in the summer, both viruses were inactivated within 7 d after addition to freshly sludge-amended soil. Soil temperature averaged 33 °C. Soil moisture content varied from near saturation at the start to dryness 2 d later, followed by saturation toward the end of the study by summer thundershowers.

According to a USEPA report (USEPA 1985), there is little evidence

linking groundwater contamination with sludge-amended soils, although few field studies have been conducted.

Studies in which laboratory-grown viruses have been used have, for the most part, yielded mixed results relative to virus movement through soil. The current hypothesis is that viruses in sludge are adsorbed to sludge flocs and, when sludge is amended to soil, these viruses remain in the sludge: soil matrix and are not easily mobilized. In support of this hypothesis, Damgaard-Larsen et al. (1977) studied the survival and movement of enteroviruses in field lysimeters in Sweden. Lysimeters were filled with soil and then amended with sewage sludge seeded with laboratory-grown poliovirus and echovirus. These studies were conducted during winter when virus survival was prolonged. Results revealed that neither of these viruses was recovered from the leachate. However, viruses were recovered from the fraction containing the sludge:soil matrix for up to 6 mon after initial seeding. This led to the conclusion that viruses are effectively retained in the sludge:soil matrix.

Pancorbo et al. (1988) studied poliovirus transport in sludge-amended soil using soil columns. Poliovirus was added to sewage sludge, which was then conditioned using chemicals or polyelectrolyte solutions. The sludge was dewatered and then applied to columns containing fine sandy loam soils. The columns were leached with distilled water for up to 10 pore volumes to simulate rainfall. These investigators also failed to demonstrate migration beneath the sludge:soil layer.

In both studies, the investigators selected polio and echovirus. The problem with using these viruses is that both are highly adsorbed in soil, regardless of the suspending medium (Hurst et al. 1980a). When wastewater effluent seeded with poliovirus and echovirus was applied to land, Hurst et al. (1980b) found that maximum downward poliovirus migration was 5–10 cm in 5 d and the maximum downward migration distance of echovirus was approximately 100-fold less under the same conditions. In contrast, Gerba and Bitton (1984) reported that coxsackie B3 virus was able to migrate 18.3 m when sewage effluent was applied to land used for artificial groundwater recharge. Downward migration from sludge-amended soils using viruses that adsorb poorly to soil like group B coxsackie has not been studied.

In summary, virus migration from sludge-amended soil appears to be limited, but it is unclear if the reasons for this are that viruses are adsorbed to the sludge flocs, soil, or both. Only a limited number of virus groups have been studied to date.

B. Indicator Bacteria

Bacterial die-off is influenced by many of the same factors as virus inactivation, with the addition of the availability of nutrients playing a role. Temperature, pH, moisture and nutrient supply have the greatest impact on enteric bacterial survival (Gerba et al. 1975). Antagonism by competing

microflora may play a significant role, but this is difficult to quantitate. Like most enteric organisms, a lower temperature increases survival time in the soil environment (Crane and Moore 1984); however, freezing and thawing conditions are detrimental (Kibbey et al. 1978.) Extremes of pH are also detrimental to bacterial survival (Kibbey et al. 1978; Hudson and Fennel 1980). Generally, a near-neutral pH environment favors extended bacterial survival (McFeters and Stuart 1972). Beard (1940) found that *Salmonella typhosa* survived best in soils between pH 6.5 and 8.0.

Moisture effects in soil systems are of major importance in bacterial decline. Kibbey et al. (1978) found that bacterial survival rates for *Streptococcus faecalis* and *Salmonella typhimurium* increased with increasing moisture content at several different temperatures. When sludges are buried, soil moisture loss is probably minimized (Crane and Moore 1984). Bacterial survival would apparently be greatest under saturated conditions (Boyd et al. 1969; Kibbey et al. 1978).

Nutrient supply, organic matter, and percolating water also affect the rate of bacterial die-off. A major reason for enteric bacterial die-off outside of the host intestinal tract is probably their inability to lower their metabolic requirements to a lower nutrient availability (Klein and Casida 1967). Mallman and Litsky (1951) felt that the organic content of sludge enhanced bacterial survival. The survival of fecal coliforms is greatly extended in organic soils over that observed in mineral soils (Tate 1978), and the regrowth of *S. typhimurium* and *E. coli* has been observed in buried feces (Temple et al. 1980).

Of all pathogenic bacteria, *Salmonella* survival has been studied most extensively (Feachem et al. 1983). They can survive in animal slurries, sludges, and soils for many months under ideal conditions (high moisture, low temperatures). *Salmonella* in sludge applied to arid land persisted for 6–7 wk (Watson 1980). Hess and Breer (1975) reported that salmonellae on grass treated with sludge could survive up to 16 mon in the climate of Switzerland, but most reported times are shorter. *Salmonella* can multiply vigorously in sterilized sludge or slurry, but under natural conditions growth is limited or strongly inhibited by the activity of microflora (Findlay 1973).

Although shigellae are among the most important pathogenic enteric bacteria, their presence and persistence in the environment have been studied far less than *E. coli* and *Salmonella*. In clean waters, survival times are typically less than 14 d at >20 °C, whereas they may survive for a few weeks below 10 °C (Feachem et al. 1983). Interestingly, McFeters et al. (1974) found that *Shigella* died more slowly in well water at 9–12 °C than the fecal bacterial indicators, *Salmonella* or *Vibrio cholerae*. No studies were found on the survival of *Shigella* in soils and sludge. A literature review on *Shigella* survival by Feachem et al. (1983) suggests that at temperatures >30 °C *Shigella* survival is less than that for *Salmonella*.

The fate of indicator bacteria after land application of anaerobically

digested sewage sludge in an arid region was reported by Pepper et al. (1991). Not only was survival on the surface horizon studied, but transport of these organisms in the unsaturated subsurface was also monitored. None of the indicator bacteria were isolated in soil samples prior to sludge amendment. However, immediately after treatment, numbers of fecal streptococci, total coliforms, and fecal coliforms were 6.1×10^6, 1.3×10^8, and 3.7×10^7 /kg of dry soil, respectively. After 5 wk, fecal streptococci had decreased to 9.6×10^3 /kg of soil, with total coliform levels at 4.6×10^6 and fecal coliform levels at 4.6×10^4 /kg of soil. However, after this time, soil temperatures decreased and, following a rainfall event, soil moisture increased and coliforms showed evidence of regrowth. After 84 d, total coliform values increased to 9.8×10^6 and fecal coliforms to 4.5×10^6 /kg. Fecal streptococci showed little regrowth after 84 d. Approximately 5 mon into the study, fecal streptococci and total coliforms were still as high as 1×10^4 and 1.0×10^5 /kg of dry soil. Seven months after the beginning of the study, soil moisture was at its lowest, and none of the three bacterial indicators were detected.

These investigators concluded that cool and moist conditions in the field could favor regrowth of the introduced indicator microorganisms unlike that for viruses. Although most indicator bacteria are mesophilic, the combination of mesophilic conditions (37–40 °C) and dry soil was detrimental to the survival of these bacteria.

A second study conducted by Pepper et al. (1991) investigated transport through the subsurface of fecal coliform indicator bacteria after land application of sewage sludge to cotton farms in the Tucson, Arizona area. Soil core samples were taken in 50-cm increments to a depth of 200 cm.

None of the indicator bacteria were detected before the land application of sludge. After application, however, fecal coliforms were detected at the 200-cm depth on the day of sludge application, but decreased approximately 2 $\log_{10}$/wk thereafter. After 7 wk, fecal coliforms were detected only in the 0- to 100-cm depth.

Vibrio cholerae appears capable of surviving for 4–10 d in soils moistened with sewage at 20–28 °C (Gerichter et al. 1975). Data are not available on the survival of *V. cholerae* in sewage sludges. Although the traditional view has been that *V. cholerae* does not survive for long periods in the environment, more recent studies suggest that prolonged survival and regrowth are possible under certain conditions (Feachem et al. 1983). Based on a literature review, Feachem et al. (1983) calculated t_{90} values (time required for the death of 90% of the original numbers of organisms) in hours for *V. cholerae* in various types of waters. They suggest that *V. cholerae* exhibits longer survival in well water and seawater than in fresh surface waters and sewage. In general, it appears that *V. cholerae* survival would be less than that of *Salmonella* at 30 °C.

Little is known about the occurrence and survival of *Yersinia enterocolitica* or *Campylobacter jejuni* in the environment. These organisms are capa-

ble of growth in foods and water at low temperatures (0–10 °C) (Bottone 1981; Highsmith et al. 1977). Dominowka and Malottke (1971) found that *Y. enterocolitica* survived 38 d in the spring and 7 d in the summer when kept outdoors in surface waters. Current evidence suggests that *Y. enterocolitica* may survive for long periods in cool, clean waters with a minimum of bacterial competition (Feachem et al. 1983).

Little information is available on the survival of *Campylobacter jejuni*, and none is available on its survival in domestic sludges or soil. Blasser et al. (1980) found that a 7 $\log_{10}$ reduction in autoclaved stream water required 5–33 d at 4 °C and 2–4 d at 25 °C. *Campylobacter* survival in stream water was >4 mon at 4 °C, but only 25 d at 25 °C (Rollins and Colwell 1986).

C. Protozoa

Many of the same factors that affect the survival of enteric viruses and bacteria in sludge-amended soils probably affect cyst viability as well. Due to the poor recovery of both *Giardia lamblia* and *Cryptosporidium* from sludge-amended soils, little if any work has been done to determine their survival and potential transport through the vadose zone to contaminate groundwater supplies. However, due to their large size relative to bacteria and viruses, cysts are unlikely to be mobile through the soil and vadose zone.

An epidemiologic study evaluating the risk factors associated with endemic giardiasis in the New England area found the use of shallow household wells for drinking water a significant risk factor (Chute et al. 1985). Numerous outbreaks of giardiasis have also occurred from surface water that was passed through sand filters. *Giardia* can penetrate a meter of fine sand (0.28-mm average diam) (Logsdon et al. 1984). From 0.1–64% of the *Giardia* cysts applied to a sand column were able to penetrate to a depth of 96 cm at operational flow rates of 0.04–0.4 m /hr. No studies were found on the expected removal of parasites by soils. Ghirose (1986) reported the isolation of protozoan cysts at several meters below the soil surface.

D. Helminths

The general consensus is that ascaris eggs are the most resistant of all enteric pathogens to adverse environmental conditions after land application (Cram 1943; Jackson et al. 1977; Meyer et al. 1978). Several researchers have observed extended survival times of ascaris eggs in soils: 4 yr (Griffiths 1978) and at least 3 yr (Jackson et al. 1977). Helminths have been observed to survive on a drying bed for 66 d (Wright et al. 1942). Soil moistures of <75% (Rudolfs et al. 1951) and 20% (Reimers et al. 1981) were lethal to *Ascaris* eggs. The lowest moisture levels at which all *Ascaris* eggs were inactivated were seasonal: 5% in fall, 7% in winter, 8% in spring, and 15% in summer (Reimers et al. 1981). Eggs were observed to survive for 60–80 d when the moisture content of the soil was <6%, and the

temperature was >40 °C (Cram 1943). Refrigerated *Ascaris* eggs have survived for >20 yr (Jackson et al. 1977).

Trichuris eggs may remain viable on soil for 6 yr (Griffiths 1978). Hookworm eggs survived 60–80 d with soil conditions of 6% moisture and >40 °C as with *Ascaris* eggs (Cram 1943). At 45 °C, hookworm larvae survive <1 hr; at 0 °C <2 wk; and at −11 °C <24 hr. Hookworms survive best in shaded sandy or loam soils covered by vegetation, protected from drying and excess wetness. Clay soil, which packs tightly, is unsuitable for hookworm survival (Metro 1983). One investigation studied the survival of *Taenia saginata* eggs in sewage, water, liquid manure, and on grass. Survival times were 16, 33, 71, and 159 d, respectively (Metro 1983).

Toxocara eggs were inactivated when the moisture content of the soil was less than 20% (Smith et al. 1980). Another study observed that moisture and temperature were responsible for the inactivation of *Toxocara* eggs. The lowest moisture levels at which all *Toxocara* eggs were inactivated were the same as those reported for *Ascaris* eggs (Reimers et al. 1981).

USEPA sponsored a study on the presence of parasites in land-applied sludges at 12 sites nationwide (Theis et al. 1978). Soils were tested only at sites that had received sludge applications for a minimum of 5 yr. In Springfield, Missouri, 50% of the sludge samples and 13% of the soil samples where sludge had been applied contained parasites. *Toxocara* was the only parasite found in the soil, whereas *Toxocara*, and to a lesser extent *Ascaris*, were found in sludge. In Hopkinsville, Kentucky, soil samples were negative, whereas 50% of sludge samples contained *Toxocara* as well as some *Ascaris*. In Frankfort, Indiana, soil samples were negative, whereas 87.5% of the sludge samples were positive with *Ascaris*, *Toxocara*, *Trichuris*, and hookworm. In Macon, Georgia, one of the 13 soil samples tested positive for *Ascaris* only. No helminths were recovered in sludge and soil samples from Kendalville, Indiana; Columbus, Indiana; Wilmington, Ohio; and Chippewa Falls, Wisconsin (Theis et al. 1978).

Anaerobically digested sludge from Oakland, California, was sprayed onto irrigated crop test plots and dryland pasture. Application rates ranged from 7.4–72.4 dry metric tons/ha. Throughout a 2-yr period soil samples from lower application rate areas were positive for helminths in 12 out of 120 samples and in 21 out of 124 samples from higher-application-rate areas. The control plot, where no sludge was directly applied, was positive for parasites in 7 out of 75 samples. This indicates either a high endemic parasite population, contamination from the test plots, or a combination of both. The parasites found, in order of frequency, were *Ascaris*, *Toxacaris*, *Toxocara*, and *Strongyloides* (Theis et al. 1978).

Because of their large size, the movement of protozoan cysts and helminth eggs would be expected to be even more limited than bacteria. Cram (1943) found no movement of *Ascaris* eggs, hookworm eggs, and *Entamoeba histolytica* cysts through a 60-cm layer of sand after application of raw settled sludge.

In another study, a glass cylinder containing a 30-cm column of sand completely removed *Taenia saginata* eggs in 3 of 4 experiments, with 99.6% removed in the fourth (Newton et al. 1949).

In a Canadian soil core experiment using ascaris seeded sludge under natural conditions, it was concluded that there was no appreciable downward movement of the parasite eggs, even in well-drained soil. After 15 d, no eggs were recovered below 2 cm. The number of eggs found on grass alone was much lower than when surface soil was included in the sample, indicating that most eggs in the sludge would remain at or near the soil surface (Metro 1979).

Studies in Russia have shown that some free-living forms of adult *Strongyloides stercoralis* penetrated to a depth of 0.3 m in soil (Shablovskaya 1963). However, no studies were found in which parasites or helminths could travel significant distances beneath sludge-amended soil.

IX. Assessment of Microbial Risks Associated with Application of Sludge to Agricultural Land

A review of the literature suggests that, in terms of risk, significant concentrations of human pathogens could be expected in sludges applied to agricultural land (Soares 1990; Pepper et al. 1991) depending on the degree of pretreatment. Most methods used in pathogen detection are not 100% efficient, and concentrations are always underestimated. In addition, methods do not exist for the detection of all pathogens that may occur in sludges. As an example, Badawy (1985) found that rotaviruses may have concentrations equal to those of enteroviruses in anaerobically digested sewage sludge. It would not be unreasonable to suggest that the actual concentrations of enteric viruses are 10–100 times the number observed experimentally.

It would also appear that many pathogens are capable of prolonged survival in sludges, especially at low temperature and high moisture conditions (Straub et al. 1992; Pepper et al. 1991). Indicator bacteria (coliforms and fecal coliforms) have survived for years in sludge and codisposal landfills (Donnelly and Scarpino 1984). The high level of organic matter probably results in the survival and growth of indicator bacteria. Bacterial pathogens such as *Salmonella* are also capable of growth in sterilized sludges (Ward et al. 1984), although this appears unlikely in digested sludges because of the large number of antagonistic bacteria. Under ideal conditions, viruses and parasites may be expected to survive for months to years, especially if the subsurface temperature is ≤ 10 °C.

The transport of pathogens from sludge-amended soils to groundwater sources is more difficult to assess. Soils with massive structure or increased clay content would be expected to slow water movement through the vadose zone and, hence, slow pathogen movement to the saturated zone. Equally

important is depth to the saturated zone. Groundwater contamination would be more of a probability at sites where the water table is less than 10 m from the surface where sludge is disposed. The true concentration of all pathogens present in sludge along with the amount disposed within a particular area is also important. Higher risks would occur if the pathogens in question do not adsorb well to sludge, soil, or both. The amount disposed per unit area is also of concern. Increased sludge and soil pH have been shown to lead to decreased adsorption of viruses and bacteria from sludge and soil (Powelson et al. 1991). Decreasing saturation in the vadose zone does lead to greater removal of pathogens (Powelson et al. 1991), but once in the saturated zone significantly less removal of microorganisms occurs unless the water table is comprised of fine textured soil.

Whether a pathogen reaches groundwater and is transported to drinking water wells depends on a number of factors, including initial concentration of the pathogens, survival of the pathogens, number of pathogens that leach from the sludge-soil interface, the degree of removal through the vadose and saturated soil zones, and the hydraulic gradient. The degree to which each of these factors influences the probability of pathogens entering groundwater cannot be determined precisely. Viruses, because of their small size, probably have the greatest potential of all pathogens for actually reaching groundwater and being transported from the site.

Although risk assessment models have been produced regarding the probability of groundwater contamination by microorganisms from the land application of municipal sewage sludge, these models have been based mostly on laboratory studies (Scarpino et al. 1988). Also, these studies are based on a few representatives from each group of pathogens of concern that have been seeded in sludge. They may not be applicable to all pathogens present in sludge or the environmental conditions to which the sludge is exposed after land application.

Recombinant DNA technology has led to the advent of sensitive and rapid detection of pathogens in the environment. The two molecular techniques that are currently being used are gene probes and nucleic amplification [polymerase chain reaction (PCR)]. Both of these techniques should provide much-needed tools for assessing the wide variety of pathogens potentially present in sewage sludge.

X. Molecular Detection Methods for Pathogens in Sludge and Soil

The methods currently used to detect pathogens in the environment have been criticized. In recent years, this criticism has focused mostly on the use of culture media (bacteria), mammalian cell lines (viruses), and fluorescent antibodies (protozoa) to detect specific pathogens in the environment. Specific media are not always available for the selective isolation of different strains of pathogens. In addition, organisms are often "injured" when intro-

duced into foreign environments such as sludges or soils, and may be viable but nonculturable (Roszak and Colwell 1987). Such viable but nonculturable cells may still be infective and yet not detected by culturable assays. Finally, detection of small numbers of pathogens in the presence of vast numbers of indigenous organisms in environmental samples requires extremely sensitive assays. The emergence of recombinant DNA technology has resulted in new detection assays with improved specificity and selectivity. In vitro amplification of deoxyribonucleic acid (DNA) via polymerase chain reaction (PCR) or ribonucleic acid via reverse transcriptase-PCR (RT-PCR) allows improved detection of bacterial and viral pathogens in environmental samples (Josephson et al. 1991; Abbaszadegan et al. 1992).

The advantages of PCR assays include (1) speed of assay; (2) increased sensitivity; and (3) the ability to detect viable but nonculturable cells, since it detects gene sequences regardless of the physiological state of the organism. Disadvantages include (1) nonspecific amplification, (2) inhibition of PCR by inorganic or organic constituents, and (3) detection of nonviable pathogens. PCR technology is still in its infancy in applications to environmental samples, and research is currently focused on two key aspects: (1) the initial processing of environmental samples and, (2) the development of specific sensitive PCR protocols for such processed samples.

DNA of bacterial pathogens in soil or sludge-amended soil can be obtained by either direct extraction of bacterial cells followed by cell lysis, or by direct lysis of cells in the environmental sample followed by DNA extraction. Steffan and Atlas (1988) extracted bacterial cells from sediments and utilized PCR to detect specific strains of *Pseudomonas* spp. Pillai et al. (1991) used a modified sucrose density centrifugation procedure to extract bacteria from soil and remove colloidal contaminants. They utilized a "double" PCR protocol and gene-specific probes to enhance the sensitivity of detection. The double PCR involves two 25 cycles of PCR with fresh deoxyribonucleic acid triphosphates (dNTP's [d adenosine triphosphate, d guanosine triphosphate, d cytosine triphosphate, and d thymine triphosphate]) and *Thermus aquaticus* (TAQ) DNA polymerase being added after the first 25 cycles of PCR. This method has proved successful for the detection of fecal coliforms in soil with detection limits of 100 ag (10^{-18}) of DNA or 1–10 colony-forming units (CFUs)/g of soil (Josephson et al. 1991). It has also been used to detect coliforms in sludge-amended soil. The major criticism of bacterial cell extraction is the selective removal of bacteria from colloidal material.

Ogram et al. (1988) pioneered the development of in situ lysis of cells in environmental samples, followed by DNA extraction and subsequent analysis. However, this technique is mostly useful on coarse textured soils low in organic material since DNA can be adsorbed by colloidal inorganic or organic material.

For viruses, specific pathogen detection systems are available for environmental samples including groundwater. Abbaszadegan et al. (1992) uti-

lized chelex resins to purify virus samples obtained from groundwater. Subsequent reverse transcriptase PCR allowed the specific detection of enteroviruses. However, reverse transcriptase PCR analysis of sludges has proved more difficult. Research is currently underway to detect enteroviruses in sludge-amended soils. Obstacles include optimization of reverse transcriptase PCR and the removal of PCR-inhibiting substances from sludge.

Overall, these novel molecular tools have the potential of becoming a presumptive test for the detection of pathogenic microorganisms in environmental samples with improved specificity and sensitivity. However, we are currently at the developmental stage and much research is needed.

XI. Conclusions

Significant numbers of pathogens exist in sludge even after stabilization and treatment. If these pathogens can remain viable for extended periods of time, groundwater sources beneath sludge disposal and land application sites may become contaminated. Pathogens may not be significantly inactivated or removed by transport through the vadose zone. Once in groundwater, they may travel significant distances from the site. For viruses and parasites, the infectious dose is low, 1–50 organisms (Gerba 1986). If the concentration of either of these pathogens exceeds 10^{-3}/mL of groundwater, there could be a significant risk of infection on an annual and lifetime basis (Gerba and Rose 1990).

Further studies are required to determine the true fate of pathogens in sludge-amended soils. Studies should be conducted to determine what factors allow pathogens to leach from the sludge:soil matrix and the concentration of these pathogens in the leachate. In most field studies, there is no mention of groundwater monitoring at these sites. In addition to providing useful information on organic and inorganic contaminants leaching from these sites, the true number of potential pathogens could be determined, rather than estimated, making it possible to forecast better risk assessment models.

Improved methods of isolation of pathogen groups from sludge and sludge-amended soil are needed. This is especially true for protozoan parasites and helminths. Recovery efficiency for both of these pathogen groups is approximately 1%. Also, better excystation procedures need to be developed for both of these groups to determine if these pathogens are still viable.

Finally, none of these steps is necessary if pathogens are destroyed at the wastewater treatment facility. Interdisciplinary research between civil engineers and microbiologists could be beneficial in the design of pilot-scale sludge treatment plants that would achieve the desired treatment goal of total pathogenic microorganism destruction. Based on successful results, this technology could be transferred and implemented at municipal wastewater-treatment facilities.

Summary

Sewage sludge is a complex mixture of organic and inorganic compounds of biological and mineral origin that are precipitated from wastewater and sewage during primary, secondary, and tertiary sewage treatment. Present in these sludges are significant numbers of microorganisms that include viral, bacterial, protozoan, fungal, and helminth pathogens. The treatment of sludge to reduce biochemical oxygen demand, solids content, and odor is not always effective in reducing numbers of pathogens. This becomes a public health concern because the infectious dose for some of these pathogens may be as low as 1 particle (virus) to 50 organisms (*Giardia*). When sludge is applied to land for agricultural use and landfill compost, these pathogens can survive from days (bacteria) to months (viruses) to years (helminth eggs), depending on environmental conditions. Shallow aquifers can become contaminated with pathogens from sludge and, depending on groundwater flow, these organisms may travel significant distances from the disposal site. Communities that rely on groundwater for domestic use can become exposed to these pathogens, leading to a potential disease outbreak. Currently, methods to determine the risk of disease from pathogens in land-disposed sludge are inadequate because the sensitivity of pathogen detection is poor. The application of recombinant DNA technology (gene probes and polymerase chain reaction) to environmental samples may provide increased sensitivity for detecting specific pathogens in land-disposed sludge and greatly improved risk assessment models for our exposure to these sources of pathogens.

References

Abbaszadegan M, Huber MS, Gerba CP, Pepper IL (1992) Detection of enteroviruses in groundwater using polymerase chain reaction. Appl Environ Microbiol (submitted for publication).

APHA (American Public Health Association) (1975) Control of communicable diseases in man, 12th ed. Washington DC.

Archer DL, Kvenberg JE (1985) Incidence and cost of foodborne diarrheal disease in the United States. J Food Prot 48:887–894.

Aserkoff B, Schroeder S, Brachman P (1970) Salmonellosis in the United States—A five year review. Am J Epidemiol 92:13–24.

Atlas RM, Bartha R (1987) Ecological aspects of biodeterioration control: soil, waste, and water management. In: Microbial ecology, 2nd ed. Benjamin/Cummings Publ Co, Menlo Park, CA, pp 367–400.

Badawy A (1985) Unpublished results. Univ Arizona, Tucson, AZ.

Bagdasar'yan GA (1964) Survival of viruses in the enterovirus group (poliovirusmyelitis, echo, coxsackie) in soil and on vegetables. J Hyg Epidemiol Microbiol Immunol 8:497–506.

Beard P (1940) Longevity of *E. typhosa* in various soils. Am J Pub Hlth 30:1077–1082.

Berg G, Berman D (1980) Destruction by anaerobic mesophilic and thermophilic

digestion of viruses and indicator bacteria indigenous to domestic sludges. Appl Environ Microbiol 39(2):361-368.
Bitton G (1980) Fate of viruses in sewage treatment plants. In: Bitton G (ed) Introduction to environmental virology. Wiley, New York, pp 122-151.
Bitton G, Pancorbo OC, Farrah SR (1984) Virus transport and survival after land application of sewage sludge. Appl Environ Microbiol 47:905-909.
Blasser MJ, Wilson FM, Wang NA (1980) Reservoirs for human campylobacteriosis. J Infect Dis 141:665-669.
Bottone EJ (1981) *Yersinia enterocolitica*. CRC Press, Boca Raton, FL.
Boyd JW, Yoshida T, Vereen LE (1969) Bacterial response to the soil environment. Sanitary Eng Paper No 5, Colorado State Univ, Fort Collins, CO.
Chute C, Smith R, Baron J (1985) Risk factors for endemic giardiasis. Am J Epidemiol 122-155.
Counts CA, Shuckrow AJ (1974) Lime stabilized sludge:Its stability and effect on agriculture land. Natl Environ Res Ctr, USEPA, Cincinnati, OH, p 87.
Cram EB (1943) The effect of various treatment processes on the survival of helminth ova and protozoan cysts in sewage. Sewage Works J 15:1119-1138.
Cramer WN, Burge WD (1975) Destruction of an indicator virus during composting of sewage sludge. In: Meeting on agronomic research for food. Knoxville, TN, p 15.
Crane SR, Moore JA (1984) Bacterial pollution of groundwater:A review. Water, Air, Soil Pollut 22:67-83.
Damgaard-Larsen S, Jensen KO, Lund E, Nissen B (1977) Survival and movement of enterovirus in connection with land disposal of sludges. Water Res 11:503-508.
D'Antonio RG, Winn RE, Taylor JP (1985) A waterborne outbreak of *Cryptosporidiasis* in normal hosts. Ann Intern Med 103:886-888.
Dominowska C, Malottke R (1971) Survival of *Yersinia* in water samples originating from various sources. Bull Inst Mar Med Gdansk 22:173-182.
Donnelly JA, Scarpino PV (1984) Isolation, characterization and identification of microorganisms from laboratory and full scale landfills. EPA-600/52-84-119, USEPA, Cincinnati, OH.
Epstein E (1973). The physical processes in the soil as related to sewage sludge aplication. In: Recycling municipal sludges and effluents on land. Nat Assoc of State Universities and Land-Grant Colleges, Washington, DC.
Erlandsen SL, Meyer EA (1984) *Giardia* and Giardiasis. Plenum Press, New York.
Feachem RG, Bradley DJ, Garelick H (1983) Sanitation and disease. In: Health aspects of excreta and waste managment. Wiley, New York.
Findlay CR (1973) Salmonellae in sewage sludge. II. Multiplication. Vet Rec 93: 102-103.
Gary GW, Hierholzer JC, Black RE (1979) Characteristics of non-cultivatable adenoviruses associated with diarrhea in infants: A new subgroup of human adenoviruses. J Clin Microbiol 10:96-103.
Geldreich EE, Fox KR, Goodrich JA, Rice EW, Clark RM, Swerdlow DL (1992) Searching for a water suply connection in the Cabool, Missouri disease outbreak of *Escherichia coli* 0157:H7. Water Res 26:1127-1137.
Gerba CP, Wallis C, Melnick JL (1975) Fate of wastewater bacteria, viruses in soil. J Irrig Drain Div, ASCE, 101:157-174.

Gerba CP, Goyal SM, Cech I, Bogdan GF (1981) Quantitative assessment of the adsorptive behavior of viruses to soils. Environ Sci Technol 15:940–944.

Gerba CP (1983). Pathogens. In: Page AL, Gleason TL, Smith JE, Iskander IK, Sommers LE (eds) Utilization of municipal wastewater and sludge on land. Univ of California, Riverside, CA, pp 147–187.

Gerba CP, Bitton G (1984) Microbial pollutants: Their survival and transport pattern to groundwater. In: Bitton G, Gerba CP (eds) Groundwater pollution microbiology. Wiley, New York, pp 65–88.

Gerba CP, Singh SN, Rose JB (1985) Waterborne viral gastroenteritis and hepatitis. CRC Crit Rev Environ Control 15:213–236.

Gerba CP (1986) Transport and fate of viruses in soils: Field studies. In: Rao C (ed) Human viruses in sediments, sludges and soils. CRC Press, Boca Raton, FL.

Gerba CP, Rose JB (1990) Viruses in source and drinking water. In: McFeters GA (ed) Drinking water microbiology: Progress and recent developments. Springer-Verlag, New York, pp 380–396.

Gerba CP (1992) unpublished.

Gerichter CB, Sechter I, Gavish I (1975) Viability of *Vibrio cholerae* biotype El Tor and cholera phage on vegetables. Israel J Med Sci 11 889–895.

Ghriose W (1986) Cornell Univ, Ithaca, New York (personal communication).

Gravaghan PD, Sykora JL, Jakubowski W, Sorber CA, Casson LW, Casson AM, Sninsky AM, Lichte MD, Keleti G (1993) Inactivation of *Giardia* by anaerobic digestion of sludge. Water Sci Technol (in press).

Griffiths HJ (1978) A handbook of veterinary parasitology of domestic animals of North America. Univ of Minnesota, Minneapolis, MN.

Hammerberg B, MacInnis GA, Hyler T (1978) *Taenia saginata cysticerci* in grazing steers in Virginia. J Am Vet Med Assoc 173:1462–1464.

Hass CN (1983) Estimation of risk due to low doses of microorganisms: A comparison of alternative methodologies. Am J Epidemiol 118:573–582.

Herwaldt BL, Craun GF, Stokes SL, Juranek DD (1991) Waterborne-disease outbreaks, 1989–1990. In: CDC surveillance summaries, Dec. 1991. Morbidity Mortality Weekly Repts 40:1–22.

Herwaldt BL, Craun GF, Stokes SL, Juranek DD (1992) Outbreaks of waterborne disease in the United States: 1989–90. J Am Water Works Assoc 84:129–135.

Hess E, Breer C (1975) Epidemiology of Salmonellae and fertilizing grassland with sewage sludge. Zentral Bakt Parasit Infekt Hyg 16:154–60.

Highsmith AK, Feeley JC, Skaliy P (1977) Isolation of *Yersinia enterocolitica* and growth in distilled water. Appl Environ Microbiol 34:745–750.

Holler C (1988) Quantitative and qualitative investigations of *Campylobacter* in a sewage treatment plant. Zbl Bakt Hyg B 185:326–339.

Huang RT, Li DR, Wei J, Huang XR, Yuan XT, Tian X (1992) Isolation and identification of hepatitis E virus in Xinjiana. China J Gen Virol 73:1143–1148.

Hudson JA, Fennel H (1980) Disposal of sewage sludge to land:Chemical and microbiological aspects of sludge to land policy. J Water Pollut Control Fed 52: 370–387.

Hurst CJ, Gerba CP, Cech I (1980a) Effects of environmental variables and soil characteristics on virus survival in soil. Appl Environ Microbiol 40:1067–1079.

Hurst CJ, Gerba CP, Lance JC, Rice RC (1980b) Survival of enteroviruses in

rapid-infiltration basins during the land aplication of wastewater. Appl Environ Microbiol 40:192–200.

Hurst CJ (1988). Fate of viruses during wastewater sludge treatment processes. CRC Crit Rev Environ Control 18:317–343.

Jackson GT, Bier JW, Rude RA (1977) Recycling of refuse into the food chain: The parasite problem. In: Sagik BP, Sorber CA (eds), Risk assessment and health effects of land application of municipal wastewater and sludges. Ctr for Appl Res and Tech, Univ of Texas, San Antonio, TX.

Jewell WJ, Kabrick RM, Spada JA (1980) Autoheated aerobic thermophilic digestion with air aeration. Municipal Environ Res Lab, R804636 USEPA, Cincinnati, OH.

Jones K, Betaieb M, Telford DR (1990a) Seasonal variation of thermophilic campylobacters in sewage sludge. J Appl Bacteriol 69:185–189.

Jones K, Betaieb M, Telford DR (1990b) Correlation between environmental monitoring of thermophilic campylobacters in sewage effluent and the incidence of *Campylobacter* infection in the community. J Appl Bacteriol 69:235–240.

Josephson KL, Pillai SD, Way J, Gerba CP, Pepper IL (1991) Fecal coliforms in soil detected by polymerase chain reaction and DNA-DNA hybridizations. Soil Sci Soc Am J 55:1326–1332.

Kayed D (1986) Methods for the isolation and detection of *Cryptosporidium* and *Giardia* cysts from stools. MS thesis, Univ of Arizona, Tucson, AZ, p 97.

Kibbey HJ, Hagedorn C, McCoy EL (1978) Use of fecal streptococci as indicators of pollution in soil. Appl Environ Microbiol 35:711–717.

Klein DA, Casida LE Jr (1967) *Escherichia coli* die-out from normal soil as related to nutrient availability and the indigenous microflora. Can J Microbiol 13:1461–1470.

Kowal NE (1985) Health effects of land application of municipal sludge. EPA 600/1-85-015, USEPA, Research Triangle Park, NC.

Lacoboni M, LeBrun T (1977) Pathogen inactivation during sludge composting. Internal reps of the County Sanitation District of Los Angeles County, May and June, 1978.

Larkin EP, Tierney JT, Sullivan R (1976) Persistence of virus on sewage irrigated vegetables. Proc Am Civil Eng J Environ Eng Div 102:29–35.

Lednar WM, Lemon SM, Kirkpatrick JW (1985) Frequency of illness associated with epidemic hepatitis A virus infection in adults. Am J Epidemiol 122:226–233.

Leong LYC (1983) Removal and inactivation of viruses by treatment processes for potable water and wastewater: A review. Water Sci Technol 15:91–114.

Logsdon GS, DeWalle FB, Hendricks DW (1984) Filtration as a barrier to passage of cysts in drinking water. In: Erlandsen EA, Meyer EA (eds) *Giardia* and Giardiasis. Plenum Press, New York, pp 287–309.

Lue-Hing C, Sedita SJ, Rao KC (1977) Viral and bacterial levels resulting from the land aplication of digested sludge. Rept No 77-21, Metropolitan Sanitary District of Greater Chicago, Chicago, IL, p 44.

Mallman W, Litsky W (1951) Survival of selected enteric organisms in various types of soil. Am J Pub Hlth 41:38–44.

McFeters GA, Stuart DG (1972) Survival of coliform bacteria in natural waters. Field and laboratory studies with membrane filter chambers. Appl Microbiol 24: 805–811.

McFeters GA, Bissonette GK, Jezeski JJ (1974) Comparative survival of indicator bacteria and enteric pathogens in well water. Appl Microbiol 27:823–829.

Melnick JL, Werner HA, Phillips CA (1978) Enteroviruses. In: Diagnostic procedures for viral, rickettsial, and chlamydial infections. American Public Hlth Assoc, Washington, DC, pp 513–578.

Melnick JL, Gerba CP (1980) Viruses in water and soil. Pub Hlth Rev 9:185–213.

Metro (Municipality of Metropolitan Seattle) (1983) Health effects of sludge land application: A risk assessment. Water Quality Div, Seattle, WA.

Meyer RB, Miller KD, Kaneshiro ES (1978) Recovery of *Ascaris* eggs from sludge. J Parasitol 64:380–383.

Morris JG Jr, Wilson R, Davis BR (1981) Non-O group 1 *Vibrio cholerae* gastroenteritis in the United States. Ann Intern Med 94:656–658.

Murphy WH, Syverton JT (1985) Absorption and translocation of mammalian viruses by plants. II. Recovery and distribution of viruses in plants. Virology 6: 623–636.

Musial CE (1985) Development of a method for the detection of *Cryptosporidium* in water and selected studies on hepatitis A virus. PhD diss, Univ of Arizona, Tucson, AZ.

Newton WL, Bennett HJ, Figgat WB (1949) Observations on the effects of various sewage treatment processes upon the eggs of *Taenia saginata*. Am J Hyg 49: 166–175.

Ogram A, Sayler GS, Gustin D, Lewis RJ (1988) DNA adsorption to soils and sediments. Environ Sci Technol 22:972–984.

Pahren H, Jakubowski W (1980) Wastewater aerosols and disease. EPA 600/9-80-028. USEPA, Cincinnati, OH.

Pancorbo OC, Bitton G, Farrah SR, Gifford GE, Overman AR (1988) Poliovirus retention in soil columns after aplication of chemical- and polyelectrolyte-conditioned dewatered sludges. Appl Environ Microbiol 54:118–123.

Pederson DC (1983) Effectiveness of sludge treatment processes in reducing levels of bacteria, viruses and parasites. In: Wallis PM, Lehman DL (eds) Biological health risks of sludge disposal to land in cold climates. Univ of Calgary Press, Calgary, Alberta, Canada, pp 9–32.

Pepper IL, Gerba CP (1989) Pathogens. In: Agricultural sludge reclamation study, part 6. Pima County Wastewater Management Dept, Tucson, AZ, pp 94–146.

Pepper IL, Gerba CP, Josephson KL, Straub TM (1991) Agricultural sludge reclamation study yearly report for 1991. Pima County Wastewater Managment Dept, Tucson, AZ.

Pillai SD, Josephson KL, Bailey RL, Gerba CP, Pepper IL (1991) Rapid method for processing soil samples for polymerase chain reaction amplification of specific gene sequences. Appl Environ Microbiol 57:2283–2286.

Powelson DK, Simpson JR, Gerba CP (1991) Effects of organic matter on virus transport in unsaturated flow. Appl Environ Microbiol 57:2192–2196.

Reimers RS, Bowman DD, Englande AJ (1981) Parasites in Southern sludges and disinfection by standard sludge treatment. EPA600/52-81-166, USEPA, Cincinnati, OH.

Rollins DM, Colwell RR (1986) Viable but nonculturable stage of *Campylobacter jejuni* in the natural aquatic environment. Appl Environ Microbiol 52:531–538.

Roszak DB, Colwell RR (1987) Survival strategies of bacteria in the environment. Microbiol Rev 51:365–379.

Rudolfs WL, Falk LL, Ragotzkie RA (1951) Contamination of vegetables grown in polluted soil. III. Field studies on *Ascaris* eggs. Sewage Indust Wastes 23:478–485.

Sattar SA, Ramia S, Westwood JCN (1976) Calcium hydroxide (lime) and the elimination of human pathogenic viruses from sewage: Studies and experimentally-contaminated (poliovirus type 1, Sabin) and pilot plant studies. Can J Pub Hlth 67(3):221–226.

Scarpino PV, Fradkin L, Clark CS, Kowal NE, Lomnitz E, Baseheart M, Peterson JM, Ward RL, Hesford M (1988) Microbiological risk assessment for land application of municipal sludge. In: Lichtenberg JJ, Winter JA, Weber CI, Fradkin L (eds) Chemical and biological characterization of municipal sludges, sediments, dredge spoils, and drilling muds. American Society for Testing Materials, Washington, DC, pp 480–488.

Shablovskaya EA (1963) Some data on the vertical migration of *Strongyloides stercoralis* in the soil. Trud: Ukrainskogo Rpublikanskogo Nauchrogo Obschestva. Parazitologov 2:74–80.

Slonka GF, Moulthrop JI, Dewhirst LW (1975) An epizootic of bovine cysticercosis. J Am Vet Med Assoc 166:678–681.

Smith GS, Kiesling HE, Ray EE, Hallford DM, Herbel DH (1980) Fate of parasites in drying beds studies. In: Bitton G, Damron GL, Edds GT, Davidson JM (eds) Sludge—health risks of land application. Ann Arbor Science, Ann Arbor, MI, p 357.

Smith HV, Rose JB (1990) Waterborne Cryptosoriddiosis. Parasitol Today 6:8–12.

Soares AC (1990) Occurrence of enteroviruses and *Giardia* cysts in sludge before and after anaerobic digestion. MS thesis, Dept of Microbiology and Immunology, Univ Arizona, Tucson, AZ.

Sorber CA, Moore BE, Johnson DE (1984) Microbiological aerosols from the application of sewage sludge to land. J Water Pollut Control Fed 56:830–836.

Sorber CA, Moore BE (1987) Survival and transport of pathogens in sludge-amended soils: A critical literature review. Project summary/USEPA rept 600/S2-87/028, USEPA, Cincinnati, OH.

Steffan RJ, Atlas RM (1988) DNA amplification to enhance detection of genetically engineered bacteria in environmental samples. Appl Environ Microbiol 54:2185–2191.

Straub TM, Pepper IL, Gerba CP (1992) Persistence of viruses in desert soils amended with anaerobically digested sewage sludge. Appl Environ Microbiol 58: 636–641.

Straub TM, Pepper IL, Gerba CP (1993) Virus survival in sewage sludge-amended desert soil. Water Sci Technol (in press).

Tate RL (1978) Cultural, environmental factors affecting the longevity of *Escherichia coli* in histosols. Appl Environ Microbiol 35:925–929.

Temple KL, Camper AK, McFeters GA (1980) Survival of two enterobacteria in feces buried in soil under field conditions. Appl Environ Microbiol 40:794–797.

Theis JH, Bolton V, Storm DR (1978) Helminth ova in soil and sludge from 12 U.S. urban areas. J Water Pollut Control Fed 50:2485–2493.

USEPA (U.S. Environmental Protection Agency) (1985) Health effects of land application of sewage sludge. EPA600/1-85/015, Research Triangle Park, NC, pp 23–26.

USEPA (1986) Development of a qualitative pathogen risk assessment methodology for sludge landfilling. Cincinnati, OH.

USEPA (1989) Standards for the disposal of sewage sludge. Fed Regist 54:5746–5906.

Wallis PM, DL Lehmann (1983) The disposal of sewage sludge to land. In: Wallis PM, Lehmann DL (eds) Biological health risks of sludge disposal to land in cold climates. Univ of Calgary Press, Calgary, Alberta, Canada, pp 1–8.

Ward RL, Ashley CS (1977) Inactivation of enteric viruses in wastewater sludge through dewatering by evaporation. Appl Environ Microbiol 34:564–570.

Ward RL, Ashley CS (1978) Heat inactivation of enteric viruses in dewatered wastewater sludge. Appl Environ Microbiol 34(5):564–570.

Ward RL, Mahler RJ (1982) Uptake of bacteriophage f2 through plant roots. Appl Environ Microbiol 43:1098–1103.

Ward RL, McFeters GA, Yeager JG (1984) Pathogens in sludge: Occurrence, inactivation and potential for regrowth. Sandia Rept DAND83-0557, TTC-0428, UC-71, Sandia Natl Labs, Albuquerque, NM.

Watson D (1990) The survival of *Salmonellae* in sewage sludge aplied to arable land. J Water Pollut Control Fed 79:11–18.

Wellings FA, Lewis AL, Mountain CW (1976) Demonstration of solids-associated virus in wastewater and sludge. Appl Environ Microbiol 38:694–701.

WHO (World Health Organization) (1981) The risk to health of microbes in sewage sludge applied to land. Euro Repts and Studies No 54, Copenhagen, Denmark.

Williams FP, Akin EW (1986) Waterborne viral gastroenteritis. J Am Water Works Assoc 78:34–39.

Wright WH, Cram EB, Nolan MO (1942) Preliminary observations on the effect of sewage treatment processes on the ova and cysts of intestinal parasites. Sewage Works J 14:1274–1280.

Yeager JG, O'Brien RT (1979) Enterovirus inactivation in soil. Appl Environ Microbiol 38:694–701.

Manuscript received December 2, 1992; accepted January 22, 1993.