Chapter 2
Sewage Sludge Pathogens

2.1 What are Pathogens?

A pathogen is an organism or substance capable of causing disease. The Part 503 regulation only discusses pathogenic organisms, and throughout this document, “pathogen” refers only to living organisms, except where specified. Pathogens infect humans through several different pathways including ingestion, inhalation, and dermal contact. The infective dose, or the number of a pathogenic organism to which a human must be exposed to become infected, varies depending on the organism and on the health status of the exposed individual.

Pathogens that propagate in the enteric or urinary systems of humans and are discharged in feces or urine pose the greatest risk to public health with regard to the use and disposal of sewage sludge. Pathogens are also found in the urinary and enteric systems of other animals and may propagate in non-enteric settings. However, because this document is concerned with the regulation of sewage sludge, this chapter focuses on the pathogens most commonly found in the human enteric system.

2.2 Pathogens in Sewage Sludge

What pathogens can be found in sewage sludge?

The four major types of human pathogenic (disease-causing) organisms (bacteria, viruses, protozoa, and helminths) all may be present in domestic sewage. The actual species and quantity of pathogens present in the domestic sewage from a particular municipality (and the sewage sludge produced when treating the domestic sewage) depend on the health status of the local community and may vary substantially at different times. The level of pathogens present in treated sewage sludge (biosolids) also depends on the reductions achieved by the wastewater and sewage sludge treatment processes.

The pathogens in domestic sewage are primarily associated with insoluble solids. Primary wastewater treatment processes concentrate these solids into sewage sludge, so untreated or raw primary sewage sludges have higher quantities of pathogens than the incoming wastewater. Biological wastewater treatment processes such as lagoons, trickling filters, and activated sludge treatment may substantially reduce the number of pathogens in the wastewater (EPA, 1989). These processes may also reduce the number of pathogens in sewage sludge by creating adverse conditions for pathogen survival.

Nevertheless, the resulting biological sewage sludges may still contain sufficient levels of pathogens to pose a public health and environmental concern. Part 503 Regulation thus requires sewage sludge to be treated by a Class A pathogen treatment process or a Class B process with site restrictions. These requirements prevent disease transmission. Table 2-1 lists some principal pathogens of concern that may be present in wastewater and sewage sludge. These organisms and other pathogens can cause infection or disease if humans and animals are exposed to sufficient levels of the organisms or pathogens. The levels, called infectious doses, vary for each pathogen and each host.

As mentioned in Chapter 1, one concern is the potential effect of some human pathogens on animals. Enteric viruses can cross species lines, and animal life, particularly warm blooded animals, can be affected if they are exposed to some of the pathogens found in sewage sludge. Domestic animals are protected by site restrictions which limit grazing on sludge amended land.

How could exposure to these pathogens occur?

If improperly treated sewage sludge was illegally applied to land or placed on a surface disposal site, humans and animals could be exposed to pathogens directly by coming into contact with the sewage sludge, or indirectly by consuming drinking water or food contaminated by sewage sludge pathogens. Insects, birds, rodents, and even farm workers could contribute to these exposure routes by transporting sewage sludge and sewage sludge pathogens away from the site. Potential routes of exposure include:

**Direct Contact**
- Touching the sewage sludge.
- Walking through an area - such as a field, forest, or reclamation area - shortly after sewage sludge application.
- Handling soil from fields where sewage sludge has been applied.
Table 2-1. Principal Pathogens of Concern in Domestic Sewage and Sewage Sludge

<table>
<thead>
<tr>
<th>Organism</th>
<th>Disease/Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>Salmonellosis (food poisoning), typhoid fever</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td>Bacillary dysentery</td>
</tr>
<tr>
<td>Yersinia sp.</td>
<td>Acute gastroenteritis (including diarrhea, abdominal pain)</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>Cholera</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>(pathogenic strains)</td>
<td></td>
</tr>
<tr>
<td><strong>Enteric Viruses</strong></td>
<td></td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>Infectious hepatitis</td>
</tr>
<tr>
<td>Norwalk and Rotavirus</td>
<td>Epidemic gastroenteritis with severe diarrhea</td>
</tr>
<tr>
<td>Norwalk-like viruses</td>
<td>Acute gastroenteritis with severe diarrhea</td>
</tr>
<tr>
<td><strong>Enteroviruses</strong></td>
<td></td>
</tr>
<tr>
<td>Polioviruses</td>
<td>Poliomyelitis</td>
</tr>
<tr>
<td>Coxsackieviruses</td>
<td>Meningitis, pneumonia, hepatitis, fever, cold-like symptoms, etc.</td>
</tr>
<tr>
<td>Echoviruses</td>
<td>Meningitis, paralysis, encephalitis, fever, cold-like symptoms, diarrhea, etc.</td>
</tr>
<tr>
<td>Reovirus</td>
<td>Respiratory infections, gastroenteritis</td>
</tr>
<tr>
<td>Astroviruses</td>
<td>Epidemic gastroenteritis</td>
</tr>
<tr>
<td>Caliciviruses</td>
<td>Epidemic gastroenteritis</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>Acute enteritis</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>Giardiasis (including diarrhea, abdominal cramps, weight loss)</td>
</tr>
<tr>
<td>Balantidium coli</td>
<td>Diarrhea and dysentery</td>
</tr>
<tr>
<td>Toxoplasma gondii</td>
<td>Toxoplasmosis</td>
</tr>
<tr>
<td><strong>Helminth Worms</strong></td>
<td></td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>Digestive and nutritional disturbances, abdominal pain, vomiting, restlessness</td>
</tr>
<tr>
<td>Ascaris suum</td>
<td>May produce symptoms such as coughing, chest pain, and fever</td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>Abdominal pain, diarrhea, anemia, weight loss</td>
</tr>
<tr>
<td>Toxocara canis</td>
<td>Fever, abdominal discomfort, muscle aches, neurological symptoms</td>
</tr>
<tr>
<td>Taenia saginata</td>
<td>Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances</td>
</tr>
<tr>
<td>Taenia solium</td>
<td>Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances</td>
</tr>
<tr>
<td>Necator americanus</td>
<td>Hookworm disease</td>
</tr>
<tr>
<td>Hymenolepis nana</td>
<td>Taeniasis</td>
</tr>
</tbody>
</table>


**Indirect Contact**

- Inhaling microbes that become airborne (via aerosols, dust, etc.) during sewage sludge spreading or by strong winds, plowing, or cultivating the soil after application.

- Consumption of pathogen-contaminated crops grown on sewage sludge-amended soil or of other food products that have been contaminated by contact with these crops or field workers, etc.

- Consumption of pathogen-contaminated milk or other food products from animals contaminated by grazing in pastures or fed crops grown on sewage sludge-amended fields.

- Ingestion of drinking water or recreational waters contaminated by runoff from nearby land application sites or by organisms from sewage sludge migrating into ground-water aquifers.

- Consumption of inadequately cooked or uncooked pathogen-contaminated fish from water contaminated by runoff from a nearby sewage sludge application site.

- Contact with sewage sludge or pathogens transported away from the land application or surface disposal site by rodents, insects, or other vectors, including grazing animals or pets.

The purpose of the Part 503 regulation is to place barriers in the pathway of exposure either by reducing the number of pathogens in the treated sewage sludge (biosolids) to below detectable limits, in the case of Class A treatment, or, in the case of Class B treatment, by preventing direct or indirect contact with any pathogens possibly present in the biosolids.

Each potential pathway has been studied to determine how the potential for public health risk can be alleviated. The references listed at the end of this chapter include some of the technical writings which summarize the research on which the Part 503 regulation is based.

For example, the potential for public health impacts via inhalation of airborne pathogens was examined. Pathogens may become airborne via the spray of liquid biosolids from a splash plate or high-pressure hose, or in fine particulate dissemination as dewatered biosolids are applied or incorporated. While high-pressure spray applications may result in some aerosolization of pathogens, this type of equipment is generally used on large, remote sites such as forests, where the impact on the public is minimal. Fine particulates created by the application of dewatered biosolids or the incorporation of biosolids into soil may cause very localized fine particulate/dusty conditions, but particles in dewatered biosolids are too large to travel far, and the fine particulates do not spread beyond the immediate area. The activity of applying and incorporating biosolids may create dusty conditions. However, the biosolids are moist materials and do not add to the dusty conditions, and by the time biosolids have dried sufficiently to create fine particulates, the pathogens have been reduced (Yeager and Ward, 1981).

The study of each pathway and the potential for public health risk resulted in site restrictions that are protective of public health and the environment and that must be followed when Class B biosolids are land applied. While the site restrictions provided in the Part 503 rule are sufficient to protect the public from health impacts, workers exposed to Class B biosolids might benefit from several additional precautions. For example, dust masks should be worn for the spreading of dry materials, and workers should wash...
their hands carefully after working with sewage sludge or biosolids. Other recommended practices for workers handling biosolids or sewage sludge include:

- Wash hands before eating, drinking, smoking or using the restroom.
- Use gloves when touching biosolids or sewage sludge or surfaces exposed to biosolids or sewage sludge.
- Remove excess sewage sludge or biosolids from shoes prior to entering an enclosed vehicle.
- Keep wounds covered with clean, dry bandages.
- If contact with biosolids or sewage sludge occurs, wash contact area thoroughly with soap and water.

Table 2-2 shows the various pathways of exposure and how the process requirements and site restrictions of the Part 503 regulation protect public health for each pathway.

2.3 General Information on Pathogens

The EPA has attempted, through this and other documents, to provide the public with a broad understanding of the risk assessment and scientific basis of the Part 503 regulation. The regulation is based on the results of extensive research and experience with land application of treated sewage sludge (biosolids). However, as for all regulations, proper interpretation and implementation of the regulation are the most important aspects of protecting public health and the environment.

Biosolids preparers should have a basic knowledge of microbiology so that they can:

- Understand the goals of the Part 503 regulation and what is expected to meet the requirements
- Address questions regarding pathogens and the protection of public health and the environment
- Design appropriate testing/sampling programs to meet the Part 503 requirements
- Make informed decisions about laboratory and analytical methodology selection

This section outlines some of the generic issues of pathogen testing and quantification. References related to these issues are listed at the end of this chapter as well as in Chapter 12. Other chapters discuss sampling and sample preservation as well as meeting the Part 503 requirements in more detail.

Survivability of Pathogens

Wastewater generally contains significantly high concentrations of pathogens which may enter the wastewater system from industries, hospitals, and infected individuals. The wastewater treatment process tends to remove pathogens from the treated wastewater, thereby concentrating the pathogens in the sewage sludge. Like any other living organisms, pathogens thrive only under certain conditions. Outside of these set conditions, survivability decreases. Each pathogen species has different tolerance to different conditions; pathogen reduction requirements are therefore based on the need to reduce all pathogenic populations. Some of the factors which influence the survival of pathogens include pH, temperature, competition from other microorganisms, sunlight, contact with host organisms, proper nutrients, and moisture level.

The various Class A and Class B pathogen reduction processes as well as the site restrictions for the land appli-

<table>
<thead>
<tr>
<th>Pathways</th>
<th>Part 503 Required Site Restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handling soil from fields where sewage sludge has been applied</td>
<td>No public access* to application sites until at least 1 year after Class B biosolids application.</td>
</tr>
<tr>
<td>Handling soil or food from home gardens where sewage sludge has been applied</td>
<td>Class B biosolids may not be applied on home gardens.</td>
</tr>
<tr>
<td>Inhaling dust**</td>
<td>No public access to application sites until at least 1 year after Class B biosolids application.</td>
</tr>
<tr>
<td>Walking through fields where sewage sludge has been applied*</td>
<td>Site restrictions which prevent the harvesting of crops until environmental attenuation has taken place.</td>
</tr>
<tr>
<td>Consumption of crops from fields on which sewage sludge has been applied</td>
<td>No animal grazing for 30 days after Class B biosolids have been applied.</td>
</tr>
<tr>
<td>Ingestion of water contaminated by runoff from fields where sewage sludge has been applied</td>
<td>Class B biosolids may not be applied within 10 meters of any waters in order to prevent runoff from biosolids amended land from affecting surface water.</td>
</tr>
<tr>
<td>Ingestion of inadequately cooked fish from water contaminated by runoff from fields where sewage sludge has been applied</td>
<td>Class B biosolids may not be applied within 10 meters of any waters in order to prevent runoff from biosolids amended land from affecting surface water.</td>
</tr>
<tr>
<td>Contact with vectors which have been in contact with sewage sludge</td>
<td>All land applied biosolids must meet one of the Vector Attraction Reduction options (see Chapter 8).</td>
</tr>
</tbody>
</table>

*Public access restrictions do not apply to farm workers. If there is low probability of public exposure to an application site, the public access restrictions apply for only 30 days. However, application sites which are likely to be accessed by the public, such as ballfields, are subject to 1 year public access restrictions.

**Agricultural land is private property and not considered to have a high potential for public access. Nonetheless, public access restrictions still are applied.
cation of Class B biosolids are based on research regarding the survivability of pathogens under specific treatment conditions. Table 2-3 shows a comparison of the survival of bacteria, viruses, and parasites in different sewage sludge treatments. Table 2-4 shows the survival time of various pathogens on soil or plant surfaces after land application of biosolids.

**Identification of Pathogens**

Some of the pathogens of concern that appear in domestic sewage and sewage sludge are shown in the photographs on pages 12 and 13. These include ascarids (Ascaris lumbricoides and Toxocara), whipworms (Trichuris sp.), tapeworms (Hymenolepis sp. and Taenia sp.), amoeba (Entamoeba coli), and giardia (Giardia lamblia). As shown in these photographs, several color staining procedures are needed to identify the organisms and the different structures within the organisms. The photograph of Giardia lamblia depicts specimens stained with Lugol’s iodine solution, showing two nuclei, a median body, and axonemes in each. In addition, scientists use a blue filter when photographing the pathogenic organisms through a microscope. This filter is necessary to show the natural color of the organisms.

**What Units are Used to Measure Pathogens?**

Density of microorganisms in Part 503 is defined as number of microorganisms per unit mass of total solids (dry weight). Ordinarily, microorganism densities are determined as number per 100 milliliters of wastewater or sewage sludge. While the use of units of volume is sensible for wastewater, it is less sensible for sewage sludge. Many microorganisms in sewage sludge are associated with the solid phase. When sewage sludge is diluted, thickened, or filtered, the number of microorganisms per unit volume changes markedly, whereas the number per unit mass of solids remains almost constant. This argues for reporting their densities as the number present per unit mass of solids, which requires that sewage sludge solids content always be determined when measuring microorganism densities.

A second reason for reporting densities per unit mass of total solids is that biosolids application to the land is typically measured and controlled in units of mass of dry solids per unit area of land. If pathogen densities are measured as numbers per unit mass of total solids, the rate of pathogen application to the land is directly proportional to the mass of dry biosolids applied.

**Different Methods for Counting Microorganisms**

The methods and units used to count microorganisms vary depending on the type of microorganism. Viable helminth ova are observed and counted as individuals (numbers) under a microscope. Viruses are usually counted in plaque-forming units (PFU). Each PFU represents an infection zone where a single infectious virus has invaded and infected a layer of animal cells. For bacteria, the count is in colony-forming units (CFU) or most probable number (MPN). CFU is a count of colonies on an agar plate or filter disk. Because a colony might have originated from a clump of bacteria instead of an individual, the count is not necessarily a count of separate individuals. MPN is a statistical estimate of numbers in a sample. The sample is diluted at least once into tubes containing nutrient medium. The tubes are maintained under conditions favorable for bacterial growth. The original bacterial density in the sample is estimated based on the number of tubes that show growth and the level of dilution in those tubes.

**Part 503 Density Limits**

Under Part 503, the density limits for the pathogens are expressed as numbers of PFUs, CFUs, or MPNs per 4 grams dry weight sewage sludge. This terminology came about because most of the tests started with 100 ml of sewage sludge which typically contained 4 grams of sewage sludge solids. Also, expressing the limits on a “per gram” basis would have required the use of fractions (i.e., 0.25/g or 0.75/g). Density limits for fecal coliforms, the indicator organisms, however, are given on a “per gram” basis because these organisms are much more numerous than pathogens.

**2.4 Protecting Public Health - The Part 503**

The Part 503 regulation protects public health by limiting the potential for public exposure to pathogens. This is

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**Table 2-3. Summary of the Effects of Sewage Sludge Treatment on Pathogens (Log Reductions Shown*)**

<table>
<thead>
<tr>
<th>PSRP Treatment</th>
<th>Bacteria</th>
<th>Viruses</th>
<th>Parasites (protozoa and helminths)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic Digestion</td>
<td>0.5-4.0</td>
<td>0.5-2.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Aerobic Digestion</td>
<td>0.5-4.0</td>
<td>0.5-2.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Composting (PSRP)</td>
<td>2.0-4.0</td>
<td>2.0-4.0</td>
<td>2.0-4.0</td>
</tr>
<tr>
<td>Air Drying</td>
<td>0.5-4.0</td>
<td>0.5-4.0</td>
<td>0.5-4.0</td>
</tr>
<tr>
<td>Lime Stabilization</td>
<td>0.5-4.0</td>
<td>4.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*A 1-log reduction (10-fold) is equal to a 90% reduction. Class B processes are based on a 2-log reduction.

**Table 2-4. Survival Times of Pathogens in Soil and on Plant Surfaces**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Soil Absolute Maximum</th>
<th>Common Maximum</th>
<th>Plants Absolute Maximum</th>
<th>Common Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>1 year</td>
<td>2 months</td>
<td>6 months</td>
<td>1 month</td>
</tr>
<tr>
<td>Viruses</td>
<td>1 year</td>
<td>3 months</td>
<td>2 months</td>
<td>1 month</td>
</tr>
<tr>
<td>Protozoan cysts</td>
<td>10 days</td>
<td>2 days</td>
<td>5 days</td>
<td>2 days</td>
</tr>
<tr>
<td>Helminth ova</td>
<td>7 years</td>
<td>2 years</td>
<td>5 months</td>
<td>1 month</td>
</tr>
</tbody>
</table>

*a For survival rates, see Sorber and Moore (1986).
*b Absolute maximum survival times are possible under unusual conditions such as consistently low temperatures or highly sheltered conditions (e.g., helminth ova below the soil in fallow fields) (Kowal, 1985).
*d Little, if any, data are available on the survival times of Giardia cysts and Cryptosporidium oocysts.
Source: Kowal, 1985.*
Ascaris lumbricoides (or var. suum) eggs, 66 µm, from anaerobically digested sludge. Two-cell stage. (Photos on this page courtesy of Fox et al., 1981)

Trichuris sp. egg, 60 µm from anaerobically digested sludge.

Toxocara sp. egg, 90 µm from raw sewage.
*Taenia* sp. ovum. (Photo courtesy of Fox et al., 1981)

*Giardia lamblia* cysts. (Photo courtesy of Frank Schaefer, U.S. EPA, National Risk Management Research Laboratory, Cincinnati, Ohio)

*Hymenolepis* (tapeworm) ova. (Photo courtesy of Fox et al., 1981)

Preparing compost for pathogen analysis. (Photo courtesy of U.S. Department of Agriculture, Beltsville, Maryland)

*Entamoeba coli* cysts, 15 µm from anaerobically digested sludge. (Photo courtesy of Fox et al., 1981)
accomplished through treatment of the sewage sludge or through a combination of sewage sludge treatment and restrictions on the land application site that prevent exposure to the pathogens in the biosolids and allow time for the environment to reduce the pathogens to below detectable levels. The Part 503 vector attraction reduction requirements also help reduce the spread of pathogens by birds, insects, and other disease carriers (i.e., vectors) by requiring that all sewage sludge that is to be land applied undergo vector attraction reduction.

The Part 503 regulation also establishes the analytical protocol for pathogen analysis. More information on the quantification of pathogens and how pathogen reduction is measured is included in Chapter 10 and in the Appendices.

**Reducing the Number of Pathogens**

Pathogen reduction can be achieved by treating sewage sludge prior to use or disposal and through environmental attenuation. Many sewage sludge treatment processes are available that use a variety of approaches to reduce pathogens and alter the sewage sludge so that it becomes a less effective medium for microbial growth and vector attraction (Table 2-5). Processes vary significantly in their effectiveness. For example, some processes (e.g., lime stabilization) may effectively reduce bacteria and viruses but have little or no effect on helminth eggs. The effectiveness of a particular process can also vary depending on the conditions under which it is operated. For example, the length of time and the temperature to which sewage sludge is heated is critical to the effectiveness of heat-based treatment processes.

Part 503 lists sewage sludge treatment technologies that are judged to produce biosolids with pathogens sufficiently reduced to protect public health and the environment. The regulation also allows the use of any other technologies that produce biosolids with adequately reduced pathogens as demonstrated through microbiological monitoring. The Part 503 establishes two classifications of biosolids based on the level of pathogen reduction the biosolids have undergone. Class A biosolids are treated to the point at which pathogens are no longer detectable. For Class B biosolids, a combination of treatment and site restrictions are designed to protect public health and the environment.

**Monitoring Indicator Species**

Sewage sludge may contain numerous species of pathogenic organisms, and analyzing for each species is not practical. The microbiological requirements of the Part 503 are therefore based on the use of an indicator organism for the possible presence of pathological bacteria and both the representative and the hardiest of known species for viruses and helminths to represent the larger set of pathogenic organisms. The indicator and representative organ-

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**Table 2-5. General Approaches to Controlling Pathogens and Vector Attraction in Sewage Sludge**

<table>
<thead>
<tr>
<th>Approach</th>
<th>Effectiveness</th>
<th>Process Example&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application of high temperatures (temperatures may be generated by chemical, biological, or physical processes).</td>
<td>Depends on time and temperature. Sufficient temperatures maintained for sufficiently long time periods can reduce bacteria, viruses, protozoan cysts, and helminth ova to below detectable levels. Helminth ova are the most resistant to high temperatures.</td>
<td>Composting (using biological processes to generate heat). Heat drying and heat treatment (use physical processes to generate heat, e.g., hot gases, heat exchangers). Pasteurization (physical heat, e.g., hot gases, heat exchangers). Aerobic digestion (biological heat)&lt;sup&gt;b&lt;/sup&gt; Anaerobic digestion (physical heat)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Application of radiation</td>
<td>Depends on dose. Sufficient doses can reduce bacteria, viruses, protozoan cysts, and helminth ova to below detectable levels. Viruses are most resistant to radiation.</td>
<td>Gamma and high-energy electron beam radiation.</td>
</tr>
<tr>
<td>Application of chemical disinfectants</td>
<td>Substantially reduces bacteria and viruses and vector attraction. Probably reduces protozoan cysts. Does not effectively reduce helminth ova unless combined with heat.</td>
<td>Lime stabilization</td>
</tr>
<tr>
<td>Reduction of the sewage sludge's volatile organic content (the microbial food source).</td>
<td>Reduces bacteria. Reduces vector attraction.</td>
<td>Aerobic digestion&lt;br&gt; Anaerobic digestion&lt;br&gt; Composting&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Removal of moisture from the sludge</td>
<td>Reduces viruses and bacteria. Reduces vector attraction as long as the sewage sludge remains dry. Probably effective in destroying protozoan cysts. Does not effectively reduce helminth ova unless combined with other processes such as high temperature.</td>
<td>Air or heat drying</td>
</tr>
</tbody>
</table>

<sup>a</sup>See Chapters 6 and 7 for a description of these processes. Many processes use more than one approach to reduce pathogens.

<sup>b</sup>Effectiveness depends on design and operating conditions.
isms are ones that have been found to respond to treatment processes and environmental conditions in a manner similar to other organisms. Monitoring the levels of these organisms, therefore, provides information about the survival of the larger group.

For example, for helminth ova, tests are employed to determine their presence and viability. The only helminth ova viability that can be determined is that of Ascaris sp. Ascaris is the hardest of known helminths; thus, if conditions are such that it cannot survive, it is not possible for other helminth species (Toxocara, Trichuris, and Hymenolepis) to survive.

For viruses, a test is available that simultaneously monitors for several enterovirus species (a subset of enteric viruses - see Table 2-1), which are presumed to be good representatives for other types of enteric viruses.

Salmonella sp. are bacteria of great concern as well as good representatives of reduction of other bacterial pathogens because they are typically present in higher densities than are other bacterial pathogens and are at least as hardy.

Fecal coliforms are enteric bacteria that are used as indicators of the likelihood of the presence of bacterial pathogens. Although fecal coliforms themselves are usually not harmful to humans, their presence indicates the presence of fecal waste which may contain pathogens. These bacteria are commonly used as indicators of the potential presence of pathogens in sewage sludges. They are abundant in human feces and therefore are always present in untreated sewage sludges. They are easily and inexpensively measured, and their densities decline in about the same proportion as enteric bacterial pathogens when exposed to the adverse conditions of sludge processing (EPA, 1992).

In the case of Class B biosolids, the microbiological limit for meeting Alternative 1 is 2 million MPN fecal coliforms per gram dry weight. Because untreated sewage sludge generally contains up to 100 million MPN fecal coliforms per gram dry weight, this limit assumes an approximate 2-log reduction in the fecal coliform population. Studies of anaerobic or aerobic digestion of sludges have shown that the corresponding reduction in the pathogens population will be significant and sufficient so that environmental attenuation can reduce pathogen levels to below detection limit within the time period of site restrictions (Farrell et al. 1985; Martin et al. 1990).

For some processes, fecal coliforms may be an overly conservative indicator. Because bacteria may proliferate outside of a host, reintroduction of fecal coliforms into treated biosolids may result in their growth. Concentrations may exceed the Class A fecal coliform limit even though pathogens are not present. In these cases, because fecal coliforms themselves are not a concern, testing directly for Salmonella sp. as an indicator of pathogen survival is permissible. Another issue with fecal coliforms is that the tests for these bacteria may overestimate the number of coliforms from human species. This is of particular concern when additives such as wood chips or other bulking agents have been added to biosolids (Meckes, 1995). In this case also, it is advisable to test directly for Salmonella sp.

It must however be noted that high counts of fecal coliforms may also indicate that a process is not being operated correctly. While a preparer may meet the regulatory requirements by testing for and meeting the regulatory limits for Salmonella sp., it is recommended that the pathogen reduction process be reviewed to determine at what point fecal coliforms are potentially not being reduced or are being reintroduced into treated biosolids, and ensure that process requirements are being fulfilled.

**Regrowth of Bacteria**

One of the primary concerns for biosolids preparers is regrowth of pathogenic bacteria. Some bacteria are unique among sewage sludge pathogens in their ability to multiply outside of a host. The processes outlined in the Part 503 regulation and in this document have been demonstrated to reduce pathogens, but even very small populations of certain bacteria can rapidly proliferate under the right conditions, for example, in sewage sludges in which the competitive bacterial populations have been essentially eliminated through treatment (see Section 4.3). Viruses, helminths, and protozoa cannot regrow outside their specific host organism(s). Once reduced by treatment, their populations do not increase. The Part 503 regulation contains specific requirements designed to ensure that regrowth of bacteria has not occurred prior to use or disposal.

**Preventing Exposure**

Exposure to pathogens in Class B biosolids is limited by restricting situations in which the public may inadvertently come into contact with biosolids and by limiting access to biosolids by vectors which may carry pathogens from the sewage sludge.

**Site Restrictions**

In the case of land application of Class B biosolids, site restrictions are sometimes required in order to protect public health and the environment. The potential pathways of exposure to Class B biosolids or to pathogens which may exist in Class B biosolids, are listed in Table 2.2 along with a description of how site restrictions impose barriers to exposure pathways. Site restrictions, discussed in detail in Chapter 5, place limits on crop harvesting, animal grazing, and public access on land where Class B biosolids have been applied.

The goal of site restrictions is to limit site activities such as harvesting and grazing until pathogens have been reduced by environmental conditions such as heat, sunlight, desiccation, and competition from other microorganisms. Table 2-3 summarizes the survival rates of four types of pathogenic organisms on soil and on plants. As shown,
helminths have the longest survival time; consequently, the duration of some of the site restrictions is based on helminth survival potential.

**Vector Attraction Reduction**

Insects, birds, rodents, and domestic animals may transport sewage sludge and pathogens from sewage sludge to humans. Vectors are attracted to sewage sludge as a food source, and the reduction of the attraction of vectors to sewage sludge to prevent the spread of pathogens is a focus of the Part 503 regulation. Vector attraction reduction can be accomplished in two ways: by treating the sewage sludge to the point at which vectors will no longer be attracted to the sewage sludge and by placing a barrier between the sewage sludge and vectors. The technological and management options for vector attraction reduction are discussed in Chapter 8.

**2.5 Frequently Asked Questions**

Because land application of biosolids has increased dramatically in the past several years, and because of some well publicized incidents of pathogen contamination (not necessarily related to biosolids), there have been many questions about the level to which public health is protected. Although it is not possible for every issue to be considered, the following section includes some of the questions which are most frequently asked. In addition, references are included at the end of this chapter and in Chapter 12.

**Can biosolids carry the pathogen that causes mad cow disease?**

It has been found that Bovine Spongiform Encephalopathy (BSE), or Mad Cow disease, is caused by a prion protein, or the resistant beta form of protein. The pathway for transmission is through the ingestion of tissue from infected animals. There has been no evidence that the BSE prion protein is shed in feces or urine. There have been no known cases of BSE in the United States, and the Food and Drug Administration (FDA) has taken various measures to prevent spread of the disease to or within the United States. For example, the primary route for infection, the use of animal carcasses in animal feed, is banned in this country. These measures have been effective, and BSE has not become a public health concern in the U.S. with regard to ingestion of beef or other exposure routes. Thus there should be no risk of BSE exposure from biosolids. (Tan, et al. 1999)

**Is there any risk of HIV infection from biosolids?**

The HIV virus is contracted through contact with blood or other body fluids of an infected individual. Feces and urine do not carry the HIV virus, but contaminated fluids may be discharged in minor amounts to the sewerage system. The conditions in the wastewater system are not favorable for the virus's survival. Separation from the host environment, dilution with water, chemicals from house- 

hold and industrial sewer discharges, and the length of time from discharge to treatment all impede the survival of the virus (WEF/U.S. EPA Fact Sheet, 1997). HIV is seldom detected in wastewater, and the additional treatment that wastewater goes through, producing an effluent and sewage sludge which undergoes treatment to become Class A or B biosolids, makes it virtually impossible that biosolids would contain the HIV virus. (Lue-Hing, et al. 1999)

Wastewater treatment workers may come into contact with contaminated objects (bandages, condoms, etc.), but common sense hygiene practices already in place at wastewater treatment plants including the use of protective clothing and gloves greatly reduce the potential for exposure. The U.S. Department of Health and Human Services stated in 1990 that “... these workers (wastewater treatment workers) have no increased potential of becoming infected by blood borne infectious agents. Therefore, medical waste discarded to the sanitary sewer is not likely to present any additional public health effects to the wastewater workers or to the general public.” (Johnson, et al. 1994)

**What is a bioaerosol?**

Bioaerosols are airborne water droplets containing microorganisms. These may include pathogenic microorganisms. Bioaerosols are a potential public health concern with regard to Class B biosolids because if pathogens are contained in the biosolids, they may become airborne and infect workers or the public through direct inhalation or through contact after settling on clothing or tools. It has been found that aerosolization of protozoa and helminths is unlikely, but bacteria or bacterial components (endotoxin) and viruses may become airborne and disperse from an application source depending on local meteorological and topographical conditions. However, Class B biosolids are rarely applied dry enough to become airborne; applying wet biosolids, particularly when the biosolids are incorporated or injected into the land, makes it highly unlikely that bioaerosols will be dispersed from land application.

The public access restrictions for land-applied Class B biosolids are based on the various pathways by which pathogens may impact public health. Site restrictions are adequate for the protection of public health, but site workers who are present during the application of Class B biosolids should follow standard hygiene precautions such as washing their hands after contacting biosolids and wearing dust masks if applying extremely dry material. More information on aerosolization of pathogens from land application can be found in the references following this chapter.

**What is Aspergillus fumigatus?**

*Aspergillus fumigatus* is a pathogenic fungus which is found in decaying organic matter such as sewage sludge, leaves, or wood. Because the fungus is heat resistant, and because sewage sludge composting facilities often use wood chips as a bulking agent, *A. fumigatus* has been associated with composting. Inhalation of *A. fumigatus* spores
may result in allergenic effects including irritation of the mucous membranes and asthma. However, *A. fumigatus* is a secondary, or opportunistic pathogen, and infection from *A. fumigatus* ("Aspergillosis") is limited to debilitated or immuno-compromised individuals. Studies of the health status of compost facility workers, the population most likely to be exposed to *Aspergillus fumigatus*, have shown any negative health impacts (Millner et al. 1994).

*A. fumigatus* is a ubiquitous fungus and has been found in homes, gardens, and offices at considerable levels. Numerous studies have been conducted to determine the level of the fungus in the areas surrounding active compost sites and compare this level to background concentrations of *Aspergillus fumigatus*. In general, it has been found that concentrations of *A. fumigatus* drop to background levels within 500-1000 feet of site activity. *A. fumigatus* is not covered in the Part 503.

There have been several incidents in which fruit has been contaminated with pathogens. Was this due to the land application of biosolids?

No. Pathogens such as *Salmonella* sp. and pathogenic strains of *E. coli* are typically associated with animal products (meat and eggs), but outbreaks have been known to occur as a result of vegetable or fruit contamination from the use of animal manures. Some of the well-publicized incidents include cases in which the consumption of fresh apple juice and cider resulted in widespread illness and the death of a child (Center for Disease Control, 1996). One case was found to be due to contamination from *E. coli* found in bovine feces, and the other was due to *Cryptosporidium* sp., also suspected to be from contact with animal manure. Other cases have involved the contamination of berries, melons, and alfalfa sprouts.

The Part 503 regulation applies only to the land application of biosolids. Education of field workers, regulation of working conditions, both domestically and abroad, and the use of animal manure products are beyond the scope of this document.

**What is the Fate of Giardia and Cryptosporidium During Sewage Sludge Treatment?**

*Giardia lamblia* and *Cryptosporidium parvum* are protozoan parasites that can infect the digestive tract of humans and other warm blooded animals. Semi-aquatic mammals can serve as hosts, transmitting the disease to humans who consume contaminated water. Domestic mammals (particularly ruminants) can serve as infective hosts and contaminate a drinking water supply. It is currently believed that at least 7% of the diarrheal cases in the United States are caused by *Cryptosporidium* sp.

West (1991) notes that human protozoan parasites such as *Cryptosporidium* sp. and *Giardia* sp. possess several traits which facilitate waterborne transmission. They can (1) be excreted in feces in large numbers during illness; (2) persist through conventional sewage treatment; (3) survive in an environmentally robust form or demonstrate resilience to inactivation while in aquatic environments; (4) be resistant to commonly used disinfectants in the treatment of drinking water; and (5) require low numbers to elicit infection in susceptible hosts consuming or exposed to contaminated water.

Stadterman et al. (1995) reported on an anaerobic digestion study which spiked *Cryptosporidium* sp. oocysts into the digester and then periodically removed samples to determine the die-off. They found that conventional anaerobic digestion produces about a 2-log removal or a better log reduction on this protozoan than it does on bacteria and viruses, but it does not reduce densities to the low values needed for Class A for this pathogen. The reported survival of some protozoa after anaerobic digestion at 35°C is a cause for concern.

Jenkins et al. (1998) reported that ammonia inactivates these oocysts, depending on the concentration. High pH processes that increase the free ammonia concentration can inactivate these oocysts (although pH by itself does little).

A conservative conclusion from the limited research performed is that Class B processes can only be expected to reduce protozoan pathogens by about a factor of ten. The restrictions written into the regulation (access control, growing only certain crops, restrictions on root crops, etc.) are necessary to prevent exposure to these pathogens. The Class A processes reduce protozoa to below detectable limits.

**References and Additional Resources**


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