Chapter 26

Land Application of Organic Residuals: Municipal Biosolids and Animal Manures

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26.1 INTRODUCTION TO ORGANIC RESIDUALS

The term "organic residuals" includes several different waste categories. Among them are the organic fraction of municipal solid waste, animal wastes or manure, and municipal biosolids that comprise the organic solids remaining after sewage treatment. In the United States, approximately 450,000 animal feeding operations (AFOs), some of which are concentrated animal feeding operations (CAFOs), collectively produce over 100 million dry tons of manure per year (Burkholder *et al.*, 2007). In contrast, approximately 16,500 municipal wastewater treatment plants operating in the U.S. produce a relatively small 7.2 million dry tons annually (NEBRA, 2007). Of these, the largest ≅3300 generate more than 92% of the total quantity of biosolids in the U.S. (NEBRA, 2007). Table 26.1 shows the amount of waste produced for each respective animal production industry. Conversely, an average 68 kg human produces approximately 37 kg of waste per year and 6.5 million dry tons per year for all municipalities combined. Both types of residuals are used beneficially for crop production through land application. Overall, animal manures are applied to about 10% of available agricultural land with greater than 90% of the total available animal manures being land applied. In contrast, only 0.1% of available agricultural land is spread with biosolids, accounting for 55% of the available biosolids (NEBRA, 2007; Brooks *et al.*, 2011). Though biosolids represent only a small fraction of total organic residuals produced, they are the most processed, most regulated, most studied and most controversial, with respect to disposal and beneficial reuse. In contrast, raw animal manures are not treated and are not regulated. In fact, certified organic farmers can utilize animal manures as a fertilizer and soil amendment, provided crops grown for human consumption are harvested at least 90 days after the last application (Organic Trade Association, 2012). The objective of this chapter is to compare and contrast microbial aspects of land application of municipal biosolids and animal manures.

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TABLE 26.1 Annual Amount of Waste Residual Produced per Industry (USEPA, 2004)

	Per Animal (1000 pounds live weight)	Total for an Individual CAFO (1000 animal units)	Total for the Industry
	Kg yr^{-1}	Ton yr^{-1}	Ton yr^{-1}
Cattle	9525	10,500	8.1×10^8
Dairy	13,607	15,000	1.9×10^8
Poultry	30,000	33,000	1.2×10^8
Swine	13,200	14,500	1.8×10^8

26.2 LAND APPLICATION OF BIOSOLIDS AND ANIMAL WASTES: A HISTORICAL PERSPECTIVE AND CURRENT OUTLOOK

Use of animal wastes and manures as a fertilizer source for agricultural crop production has been practiced since the days of the Roman Empire. During the twentieth century in both the United States and Europe, operations on small agricultural farms frequently consisted in both crop and animal production. Consequently, animal wastes were naturally land applied to enhance crop production. Although fossil fuel-based fertilizers replaced much of the use of manures following World War II, the practice continues today worldwide. The rise of locally grown, "organic" fresh produce and the increased costs associated with fuel-based fertilizers have renewed interest in manure as a fertilizer. Ten years ago, manure was considered to be a waste by-product of the animal production industry; however, it is now considered a commodity.

In the United States, land application of municipal wastewater and biosolids has been practiced for its beneficial effects and for disposal purposes since the advent of modern wastewater treatment about 160 years ago. In England in the 1850s, "sewage farms" were established to dispose of untreated sewage. By 1875, about 50 farms were utilizing land treatment in England, and many others close to other major cities in Europe. In the United States, sewage farms were established by about 1900. At this same time, primary sedimentation and secondary biological treatment was introduced as a rudimentary form of wastewater treatment, and land application of "sludges" began. It is interesting to note that prior to wastewater treatment, "sludge" *per se* did not exist. Municipal sludge in Ohio was used as a fertilizer as early as 1907.

Since the early 1970s, more emphasis has been placed on applying sludge to cropland at rates to supply adequate nutrients for crop growth (Hinesly *et al.*, 1972). In the 1970s and 1980s, many studies were undertaken to investigate the potential benefits and hazards of land application, in both the U.S. and Europe. Ultimately in 1993, U.S. federal regulations were established via the "Part 503 Sludge Rule." This document—"The Standards for the Use and Disposal of Sewage Sludge" (U.S. EPA, 1993) was designed to "adequately protect human health and the environment from any reasonably anticipated adverse effect of pollutants." As part of these regulations, two classes of treatment were defined as "Class A and Class B" biosolids, with different restrictions for land applications, based on the level of treatment. The term "biosolids" was coined in the 1990s by a University of Arizona faculty member. The distinction between sewage sludge and biosolids is described in Information Box 26.1, and it is important to note that the term biosolids implies treatment to defined levels. The requirements for Class A versus Class B biosolids are defined in Information Box 26.2.

Land application increased when restrictions were placed on "ocean dumping." By the year 2000, 60% of all biosolids were land applied in the U.S. Currently, most land application of biosolids in the U.S. utilizes Class B biosolids. However, due to public concern over potential hazards, in some areas of the U.S., land application of Class B biosolids has been banned. Thus, by 2004, only 55% of all biosolids was applied to soil for agronomic, silvicultural and/or land restoration processes. The remaining 45% was disposed of in municipal landfills or

Information Box 26.1 Definitions of Sewage Sludge and Biosolids

Sewage sludge. The solid, semisolid, or liquid residue generated during the treatment of domestic sewage in a treatment works.
Biosolids. Two different definitions have been developed:

EPA: The primarily organic solid product yielded by municipal wastewater treatment processes that can be beneficially recycled (whether or not they are currently being recycled).

National Research Council (2002): Sewage sludge that has been treated to meet the land-application standards in the Part 503 rule or any other equivalent land-application standards or practices.

incinerated (NEBRA, 2007), with about two-thirds of the non-land-applied material being landfilled. Of the total applied to soils, 74% was on farmland for agricultural purposes (NEBRA, 2007). A recent report indicates that approximately 200 million farmers worldwide grow crops in fields fertilized with human waste (IWMI, 2010).

Contrary to municipal wastewater treatment sludge, CAFOs and their manures are a relatively new advancement in egg/dairy/meat production systems. Though manure has been around since the "dawn of time," the idea of a CAFO has not. An AFO is defined as a feedlot or a facility where animals are kept for greater than 45 days; cattle grazing on pasture are exempt (U.S. EPA, 2004). CAFOs are then designated based on numerical criteria such as greater than 300 cattle or 9000 broiler chickens (U.S. EPA, 2004). Thus, all CAFOs are AFOs, but not all AFOs are CAFOs. Since the 1960s, the vast majority of animals raised for food and their products have been produced in CAFOs. This movement has led to the concentration of most food animals into less than 20% of all AFOs. For all AFO and CAFO food animal production, it has always been the responsibility of the owner to dispose of the manure, with reliance on disposal to nearby fields, thereby keeping costs low. The vast majority of AFO owners apply manure to owned lands, or rely on the sale or "giving away" of manure to other landowners. Manure land application has not been governed by any specific law or federal regulation; however, guidelines exist for suggested rates of land application manuring based on nutrient requirements, typically nitrogen or phosphorus, of the crop to be grown. Most states require nutrient management plans to be established prior to the establishment of a new CAFO. These plans essentially establish how the CAFO owner will dispose of the manure in both a quantitative (i.e., how much) and qualitative (i.e., which crop) manner.

Thus far, the level of scrutiny reserved for biosolids land application has not been similarly applied to manure. Anecdotally, the public has regarded manure as a "natural" material, and thus it has escaped intense criticism despite knowledge of pathogens, antibiotic resistant bacteria and nutrient runoff concerns that are all associated with land application of manures. In 1972, the Clean Water Act identified AFOs as potential pollutant sources, resulting in CAFO regulations being set in place in 1976. Increase in CAFO size necessitated revision of the regulations in 2008. Thus, the current U.S. EPA CAFO rule requires that CAFOs that discharge or propose to discharge waste need to apply for a permit, along with the establishment of a nutrient management plan. The rule was challenged, and now an AFO can apply for an exception provided that the manure can be appropriately stored to prevent accidental release (i.e., runoff contaminated with manure) during a 24-hour, 100-year storm event. These limits and rules are specifically designed to reduce discharge to surface water, and do not govern the land application of manure to soil, unless there is a threat of effluent runoff to surface water. Guidelines for manure land application also suggest harvest delays when using manure on "organic" marketed food crops. These guidelines suggest a delay of 90 to 120 days between land application and harvest, depending on the level of interaction between the manure/soil matrix and the food crop edible parts. Apart from these rules and guidelines, no other governing document regulates land application of manure.

Biosolids and manure are applied to agricultural and nonagricultural lands as a soil amendment because they improve the chemical and physical properties of soils, and because they contain nutrients for plant growth. Land application on agricultural land is utilized to grow food crops such as corn or wheat, and nonfood crops such as cotton. Nonagricultural land application includes forests, rangelands, public parks, golf courses and cemeteries. Biosolids and manure are also used to revegetate severely disturbed lands such as mine tailings or strip mine areas (Case Study 26.1).

26.2.1 Class A Versus Class B Biosolids

Biosolids are divided into two classes on the basis of pathogen content: Class A and Class B (Information Box 26.2). Class A biosolids are treated to reduce the presence of pathogens to below detectable levels, and can be used without any pathogen-related restrictions at the application site. Class A biosolids can also be bagged and sold to the public. Class B biosolids are also treated to reduce pathogens, but still contain detectable levels of them. Class B biosolids have site restrictions to minimize the potential for human exposure, until environmental factors such as heat, sunlight or desiccation have further reduced pathogen numbers. Class B biosolids cannot be sold or given away in bags or other containers or used at sites used by the public.

Information Box 26.2 Part 503 Pathogen Density Limits for Class A and B Biosolids

Standard Density Limits (Dry Weight)

Pathogen or Indicator

Class A

Salmonella <3 MPN/4 g total solids or
Fecal coliforms <1000 MPN/g and
Enteric viruses <1 PFU/4 g total solids and
Viable helminth ova <1/4 g total solids

Class B

Fecal coliform density <2,000,000 MPN/g total solids

Adapted from U.S. EPA (2000).

Case Study 26.1 Reclamation and Revegetation of Mine Tailings using Biosolid Amendment

Mine tailings are formed in two ways: by the initial removal of vegetation, soil, and bedrock to expose the valuable copper containing ores, and then by the disposal of the crushed rock after the ore has been removed. Typically these tailings are 30–40 meters deep. Mine tailings are not the ideal medium on which to grow plants. The crushed rock consists of large and small fragments with large spaces in between them. In addition, there is no organic material; the cation exchange capacity (CEC) is very low; the water holding capacity of the material is poor to nonexistent; and there are few macronutrients (NPK) available for the plants. Soil biota, in the form of bacteria and fungi, are only present in low numbers.

The overall objective of this study was to evaluate the efficacy of dried biosolids as a mine tailing amendment to enhance site stabilization and revegetation. Mine tailing sites were established at ASARCO Mission Mine close to Sahuarita Arizona. Site 1 (December 1998) was amended with 248 tons ha^{-1} of Class A biosolids. Site 2 (December 2000) and Site 3 (April 2006) were amended with 371 tons ha^{-1} and 270 tons ha^{-1} respectively. Site D, a neighboring native desert soil acted as a control for evaluation of soil microbial characteristics. Surface amendment of Class A biosolids showed a 4 log_{10} increase in HPCs compared to unamended tailings, with the increase being maintained for a >10-year period. Microbial activities such as nitrification, sulfur oxidation and dehydrogenase activity were also sustained throughout the study period. Finally, note that extensive revegetation of the sites occurred (Figures 26.1–26.3). 16S rRNA clone libraries obtained from community DNA suggest that mine tailings amended with biosolids achieve diversity and bacterial populations similar to native soil bacterial phyla, ten years post-application (see Case Study 19.2). Thus addition of Class A biosolids to copper mine tailings in the desert southwest increased soil microbial numbers, activity and diversity relative to unamended mine tailings. Overall, the addition of biosolids resulted in a functional soil with respect to microbial characteristics which were sustainable over a ten year period.

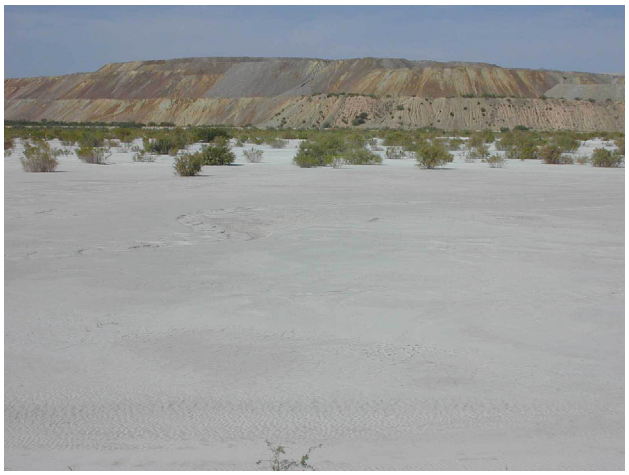

FIGURE 26.1 Mine tailings prior to biosolids amendment.

FIGURE 26.2 Mine tailings 2 years after biosolids application.

26.2.2 Methods of Land Application of Organic Residuals

26.2.2.1 Land Application of Biosolids

The method of land application of biosolids essentially depends on the percent solids contained within them, which determines whether the biosolids are liquid in nature, or a "cake" (Information Box 26.3). Figures 5.12–5.14 all illustrate methods of land application of biosolids and can be summarized as:

- Injection. Liquid biosolids are injected to a soil depth of 30 cm. Injection vehicles simultaneously disc the field. Injection processes reduce odors and bioaerosols, as well as the risk of runoff to surface waters.
- Surface application. Liquid or cake biosolids are surface applied and subsequently tilled into the soil.
- Slingers are also utilized to throw the material through the air as a means of land application.

26.2.2.2 Land Application of Animal Manures

Land application techniques for manure are far more varied than for land application of municipal biosolids. This is due, in part, to the variability associated with the various AFOs, which can include manure from cattle,

FIGURE 26.3 Mine tailings 3 years after biosolids application.

FIGURE 26.4 Liquid manure injection: coulters cut a path in the pasture with injectors applying swine liquid manure effluent below the surface in a furrow. Photo courtesy J.P. Brooks.

Information Box 26.3 Land Application Methods

% solids	Nature of biosolids	Method of application
8	Liquid	Spray application (Figure 5.12)
2	Liquid	Sprinkler system (Figure 5.13)
>20	Cake	Spreaders or slingers (Figure 5.14)

FIGURE 26.5 Subsurface banding (dark section in soil probe at 2.5" mark) with banding applicator (inset). Photo courtesy H. Tewolde.

dairy, poultry and swine industries. In addition, there are smaller operations specializing in niche foods such as ostrich, lamb and bison, which contribute to AFO manure burden. Even within a large industry such as poultry, variability is considerable, given the specific subindustries such as egg production and turkey farms. Egg layer farms typically produce liquid manure, whereas turkey producers produce a litter/fecal matter solid mixture. Each AFO produces a different kind of manure, making the standardization afforded by the USEPA Part 503 Rule a milestone which is difficult to reach. Typically prior to land application, most AFOs store manure in a shed or lagoon for a period of time, depending on season and demand. Storage type depends on solid content, with shed storage reserved for solid manure, while lagoons are utilized for liquid or slurry manure. Composting or "unofficial composting" (typically consists of long-term manure storage, without temperature monitoring or any other handling) are common pretreatments prior to land application. Likewise, in lieu of land application, some poultry producers opt to combust or burn their litter for energy production.

Manure can vary in physical characteristics from a slurry (<2% solids) to a cake (>50% solids); thus, land application can be quite varied. The microbiology associated with each manure type can also vary dramatically based on production, storage and animal management. Pasture, cotton, food crops and forage are the major crops utilizing manure. Typically, manure land application is conducted as close as possible to the producing AFO. However, given the increasing costs of traditional fertilizers, it is now easier to justify transport of manure across greater distances. As with biosolids, manure handling is based on solid content. Figures 26.4–26.6 all illustrate methods of land application of animal manures, which can be summarized as:

- Injection. Liquid manure can be injected into soil, particularly useful for row crops.
- Subsurface banding. Dried or caked poultry litter can be banded into soil. The band slowly disperses nutrients over time, which is particularly useful for row crops.

- Slinging. Dry or solid manure can be surface spread onto pasture, hay or row crops.
- Center pivot or reel-gun irrigation. Low solids liquid manure can be surface spread to pasture or hay lands.
- Surface deposition. Manure/feces are deposited on pasture lands during typical grazing periods.

26.3 POTENTIAL MICROBIAL HAZARDS ASSOCIATED WITH CLASS B BIOSOLIDS, ANIMAL MANURES AND LAND APPLICATION

Both Class B biosolids and animal manures are known to contain pathogens including bacteria and protozoan parasites. Biosolids (but typically not animal manures) also contain human pathogenic viruses. Over the past 10–15 years a variety of potential microbial hazards associated with Class B biosolids and to a lesser extent animal manures have been identified (Case Study 26.2). Many of these issues involved the potential for infection from pathogens associated with organic residues. These pathogens of concern are described in Section 26.4 and the risks associated with such pathogens are described in Section 26.5. However, in addition to pathogens, other potential hazards have centered on biological but nonpathogenic issues such as antibiotic resistant bacteria, endotoxin and prions.

FIGURE 26.6 Liquid manure application using a center pivot system and a reel gun (inset). Photo courtesy M.R. McLaughlin.

Although manure is known to contain bacterial and parasitic pathogens, there have only been a few instances where human viral pathogens have been found in manure, including hepatitis E virus in pigs and norovirus in cattle. Normally, viral pathogens are exclusive to municipal wastes. Given that manure is generally not treated, bacterial counts tend to be greater in manures than in municipal-treated biosolids. In addition to pathogens, manure is known to contain high levels of antibiotic-resistant pathogenic and commensal (i.e., normal, nonpathogenic) bacteria.

26.3.1 Antibiotic-resistant Bacteria

A major area of concern with the general public has focused on the potential for antibiotic-resistant bacteria that reside in both animal manures and biosolids, due to the potential for subsequent transfer of the resistance to pathogens. Bacteria are prokaryotic organisms with the ability to metabolize and replicate very quickly. They are also very adaptable genetically. When confronted with an antibiotic, if there is even one bacterial cell with a genetic or mutational change that confers resistance to that antibiotic, it will subsequently allow for the proliferation of antibiotic-resistant bacteria. Thus, the more that antibiotics are used,

Case Study 26.2 The University of Arizona, National Science Foundation Studies on Biosolids and Land Application

During the period 1999 through 2014 the University of Arizona was funded by the National Science Foundation to conduct studies on "Water Quality" and "Water and Environmental Technology." One of the focal areas of research included studies on potential hazards associated with Class B biosolids and land application of such material. Table 26.2 highlights some of the studies undertaken. Many of the topics evaluated were highly controversial with the general public because of the concern for potential microbial infections and/or illness due to pathogens found within Class B biosoilds. Particularly worrisome for the public was the potential for infections to occur within communities offsite, following transport of pathogens as bioaerosols, or transport via leaching into underground aquifers. However, research studies on both issues showed very limited transport by either route. Hence, exposure via such mechanisms was low, and subsequently, risks to human health were also low. Additional information on the bioaerosol studies are in Section 5.6.5. Other biological concerns included the potential for disease from endotoxin, *S. aureus* and infectious proteins known as prions. However, it was shown that neither *S. aureus* nor prions survived wastewater treatment. Finally, note that regrowth of *Salmonella* did not occur following land application, indicating that site restrictions following land application would allow for inactivation of biosolid-associated pathogens without secondary regrowth. Overall, the studies indicate that the risk of adverse health effects from biosolid amended soil is low. However, diligence is necessary and the fate and transport of all emerging microbial contaminants still need to be evaluated.

TABLE 26.2 Microbial Issues Associated with Land Application of Biosolids

Issue	Concern	Outcome of Study	References
Occurrence of *Staphylococcus aureus* in biosolids	Community infections from *S. aureus* associated with biosolids amended soil	*S. aureus* does not survive wastewater treatment	Rusin *et al.*, 2003
Aerosolized bacteria and virus	Offsite community infections from bioaerosols	Limited microbial transport via bioaerosols and negligible risk to offsite communities	Brooks *et al.*, 2005a, b; Tanner *et al.*, 2005
Endotoxin	Community exposure to aerosolized endotoxin due to endotoxin associated with biosolids	Most aerosolized endotoxin derived from soil	Brooks *et al.*, 2006; Brooks *et al.*, 2007
Groundwater contamination	Groundwater contamination with viruses following transport through soil and vadose zone	Very limited transport of viruses because virus sorb to biosolids	Chetochine *et al.*, 2006
Antibiotic-resistant bacteria (ARB)	Presence of antibiotics in biosolids will increase the numbers of ARB in soil subsequent transfer of resistance to pathogens	No increase in soil ARB	Brooks *et al.*, 2007
Salmonella	*Salmonella* regrowth in biosolids and soil following land application	*Salmonella* only regrows in Class A biosolids under saturated conditions. No regrowth in amended soil	Zaleski *et al.*, 2005a
Prions	Prion infection of animals and humans following land application of prions	Prions do not survive wastewater treatment	Miles *et al.*, 2013

TABLE 26.3 Antibiotic-resistant Bacteria, as a Percentage of Total Cultured Heterotrophic Plate Count Bacteria in Environmental and Food Samples

Sample	Antibiotic Resistant (%)			
	Ampicillin[a]	Cephalothin[a]	Ciprofloxacin[a]	Tetracycline[a]
Biosolids	4.3	21.2	1.8	1.9
Composted manure	0.0	0.3	0.0	0.3
Compost	9.7	21.8	3.4	1.2
Fresh manure	0.2	0.7	1.1	0.3
Pristine soil	8.1	10.1	3.1	2.4
Dust	4.9	7.8	8.3	11.2
Ground water	60.3	41.2	22.9	21.0
Raw chicken	47.1	60.3	0.0	0.0
Raw ground beef	16.3	8.7	2.0	3.9
Head lettuce	29.9	35.8	1.5	4.5
Shredded lettuce	14.9	10.5	0.0	0.3
Tomato	0.6	20.6	0.2	0.3

Modified from Brooks et al. *(2007).*
[a]*Ampicillin (32 μg ml*$^{-1}$*), cephalothin (32 μg ml*$^{-1}$*), ciprofloxacin (4 μg ml*$^{-1}$*) and tetracycline (16 μg ml*$^{-1}$*).*

the greater the likelihood of antibiotic-resistant strains developing. The greatest concern with antibiotic resistance is the potential for human pathogenic strains to become resistant to overused antibiotics, such that the antibiotic can no longer contain the infectious agent. As is typical in most niches, commensal bacteria tend to dominate the pathogenic bacteria at levels which are orders of magnitude greater than those pertaining to the pathogens. This creates a haven for antibiotic-resistant genes, which all have the potential to transfer to true or opportunistic pathogens. The widespread, sometimes indiscriminate, use of antibiotics has raised the questions: (1) Can antibiotic resistant genes be transferred from nonpathogenic bacteria to human pathogenic strains in the environment? (2) Can antibiotic resistance in the environment, via residual land application, be transferred to the public?

Brooks *et al.* (2006) evaluated the incidence of antibiotic-resistant bacteria (ARBs) in biosolids and a variety of other environmental samples and foodstuffs. Table 26.3 shows that Class B biosolids did not contain

unusually high numbers of ARBs, and that in fact, the relative incidence was less than that found in pristine soil. Interestingly, ARB concentrations were also lower than those found in common foodstuffs such as lettuce. Therefore, food itself could be an important route of exposure to ARBs. Rates of gene transfer in soil are thought to be a relatively infrequent event without selective pressure (Neilson *et al*., 1994), which reduces the risk of antibiotic-resistant gene transfer to human pathogenic bacteria. Finally, note that soil itself is the original source of human antibiotics.

Antibiotic use in the livestock and poultry industries has gradually increased over the past three decades in direct relation to the increasing number of CAFOs in operation. Throughout this gradual cultural shift in livestock production, the need for antibiotics has increased as stocking densities and production cycles have increased. The Union of Concerned Scientists predicted the amount of antibiotics used in the industries at up to 50 million pounds annually (Chee-Sanford *et al*., 2009), with nearly half being used as a means to increase production. The Animal Health Institute refutes this total, stating that approximately 20.5 million pounds of antibiotic are used annually with approximately one-tenth thereof used to increase production (Chee-Sanford *et al*., 2009). These discrepancies highlight how little is known regarding this topic, and how contentious these issues truly are, particularly with news cycles reporting increasing antibiotic resistance in our food supply or higher incidences of nosocomial infections. Regardless, livestock industries account for a large amount of antibiotic use in the United States. Antibiotics are used: (1) to treat infections and to prevent diseases; and (2) as a prophylactic, thus increasing production. It is with the latter that most concern or blame is associated.

In either case, as opposed to human antibiotic use, treating livestock with antibiotics is conducted in a manner that promotes the treatment of nondiseased animals. Typically, CAFO animals are not individually treated for a disease. If there is an outbreak of a disease-causing pathogen, farm managers typically react by not treating just the diseased individuals (perhaps only 100 of 20,000), but by treating the entire flock or herd. This increases the likelihood for antibiotic resistance, as resistance genes can be promoted in healthy as well as diseased members of the host population.

Brooks and McLaughlin (2009) and Brooks *et al*. (2010) described the presence of antibiotic-resistant bacteria in swine and poultry CAFOs. The presence of antibiotic-resistant bacteria in swine CAFOs appeared to be influenced by the type of management employed by the producer; specifically, the presence of younger piglets increased the amount of resistance in commensal *E. coli*. In general, younger piglets led to resistance to an extra class of antibiotics (Brooks and McLaughlin, 2009). In some instances, regulatory and media pressures have forced industries to reduce antibiotic use, as has been noted in the poultry industry. Brooks *et al*. (2010) noted the overall lack of antibiotic resistance in poultry CAFO manure, and an overall decrease among staphylococci, enterococci and *E. coli* in comparison to previous studies (Brooks *et al*., 2009).

Ultimately, the concern is for the potential movement of antibiotic-resistant bacteria and genes from the "farm to the plate." Movement from the farm to the product and ultimately the consumer remains a poorly understood area (Marshall and Levy, 2011). Three potential routes exist for the transfer to occur: (1) via consumption of undercooked food; (2) via clonal spread from the occupationally exposed; (3) or from indirect manure contamination onto fresh food crops (e.g., environmental spread). Sufficient evidence exists to support clonal spread from the occupationally exposed (Marshall and Levy, 2011), while the other two routes are poorly understood. Contamination of fresh food crops via runoff, land application of manure/biosolids or feral animals has been hypothesized as indicating potential sources of contamination (Brooks *et al*., 2012a). Antibiotic resistance phenotypes have been demonstrated to move via aerosols or runoff, though in very small amounts and over small distances from the CAFO (Brooks *et al*., 2009, 2012b; Chinivasagam *et al*., 2009). Brooks *et al*. (2009) demonstrated that runoff from plots receiving litter was more concentrated with antibiotic-resistant enterococci, which was characteristic of the litter and thus demonstrated that antibiotic resistant bacteria are transported as readily as any other bacteria.

26.3.2 Endotoxin

Another issue associated with biosolids and manure is the presence of endotoxin. Endotoxin, or lipopolysaccharide (LPS) derived from the cell wall of Gram-negative bacteria, is a highly immunogenic molecule present ubiquitously in the environment (see also Section 5.6.6) (Michel, 2003). Biosolids contain large populations of bacteria, and therefore are another potential source of endotoxin. Although most surfaces contain some traces of dust-associated endotoxin, it is primarily of concern as an aerosol, since most human endotoxin ailments are pulmonary associated (Sharif El *et al*., 2004). Exposures to aerosolized endotoxin have been studied due to occupational exposures to cotton dust, composting plants and feed houses (Castellan *et al*., 1987). Exposures to levels of endotoxin as little as 0.2 endotoxin units (EUs) per m^3 derived from poultry dust have been found to cause acute pulmonary ailments such as decreases in forced expiratory volume (Donham *et al*., 2000). Chronic effects such as asthma and chronic bronchitis have been found to be

due to exposures to endotoxin from cotton dust of as little as 10 EU per m^3 on a daily basis (Olenchock, 2001).

Endotoxin concentrations in a variety of environmental samples have been investigated, and data show that the endotoxin level in Class B biosolids is similar in magnitude to that of other wastes including animal manures and compost. Since the relevance of this to human health is via inhalation, the potential for aerosolization of endotoxin during land application of biosolids and manure has also caused concern. Brooks *et al.* (2006) showed that endotoxin values measured during biosolids application were comparable to those found in untreated agricultural soils. Therefore, aerosolization of soil particles can result in endotoxin aerosolization, regardless of whether biosolids are involved. This is not surprising since bacterial concentrations in soil routinely exceed 10^8 per gram, with a majority of bacteria being Gram negative. Soil particles containing sorbed microbes can be aerosolized and hence act as a source of endotoxin (see also Section 5.6.6).

A number of studies have investigated endotoxin in CAFOs, be it cattle, poultry or swine (Dungan and Leytem 2009; Brooks *et al.*, 2010). The majority of studies report endotoxin levels greater than those recommended for farms (Dungan, 2010). However, the majority of endotoxin associated with CAFOs has been confined to open cattle/dairy farms (Dungan, 2010), and swine and poultry interior housing (Brooks *et al.*, 2010; Dungan, 2010). For example, swine barns were found to have mean concentrations of endotoxin of 4385 EU per m^3 (Duchaine *et al.*, 2001), while composting plants ranged from 10 to 400 EU per m^3 (Clark *et al.*, 1983). Endotoxin release from open lot CAFOs (Dungan, 2010) and building exhaust fans (Brooks *et al.*, 2010) has been shown to be at levels of $\approx$800 and 100 EU m^{-3}, respectively, with rapid decreases to near background levels just beyond the point source. It can be assumed that, as with municipal biosolids land application, the majority of aerosolized endotoxin will most likely arise from the dry soil surrounding the site; however, some manure, such as dry poultry litter, will be very prone to endotoxin release. Litter endotoxin levels are approximately one order of magnitude greater than those of typical Class B biosolids (Brooks *et al.*, 2007). In all cases with endotoxin, the severity of the exposure is unknown since not all endotoxin is bioactive, and thus not all exposures are equal. Overall, land application of residuals and aerosolized endotoxin remains an area that is poorly understood by environmental microbiologists.

26.3.3 Prions

Prions are infectious proteins that can result in animal or human disease (see also Section 2.5.2.2). Transmissible spongiform encephalopathies (TSE) are a group of neurological prion diseases of mammals, which in humans include Kuru, Creutzfeldt–Jakob disease (CJD), sporadic Creutzfeldt–Jakob disease (sp CJD) and variant Creutzfeldt–Jakob disease (VCJD) (Prusiner, 2004; Miles *et al.*, 2013). Animal diseases such as bovine spongiform encephalopathy (BSE) are of particular concern. Prions have been detected in the environment at low concentrations (Nichols *et al.*, 2009), and could originate from slaughterhouse wastes. Such wastes could reach wastewater treatment plants, and therefore interest has focused on whether or not prions survive wastewater treatment. If prions survived treatment, then they could end up within biosolids, with subsequent potential exposure of animals following land application. Adding to this concern is the fact that prions are reported to be very resistant to extreme physical conditions including irradiation and heat, and chemical treatment including acids, bases and oxidizing agents (Taylor, 2000).

Within the last few years it has been reported that prions are capable of surviving a very common wastewater treatment, namely mesophilic anaerobic digestion (Kirchmayr *et al.*, 2006; Hinckley *et al.*, 2008). However, these studies utilized an immunoblot method of detection of the prions, which did not distinguish between infectious and noninfectious prions. More recently, Miles *et al.* (2013) developed an assay that only detected infectious prions. This assay utilized a standard scrapie cell assay linked to an enzyme linked immuno-spot reaction (ELISPOT) for infectious prion detection. Using this assay and miniature anaerobic digestors (Figure 26.7) the influence of various wastewater treatments on infectious prion inactivation was evaluated (Table 26.4).

These data show a quantifiable reduction of infectious prions in wastewater during the normal period of anaerobic digestion (21 days), at both mesophilic and thermophilic

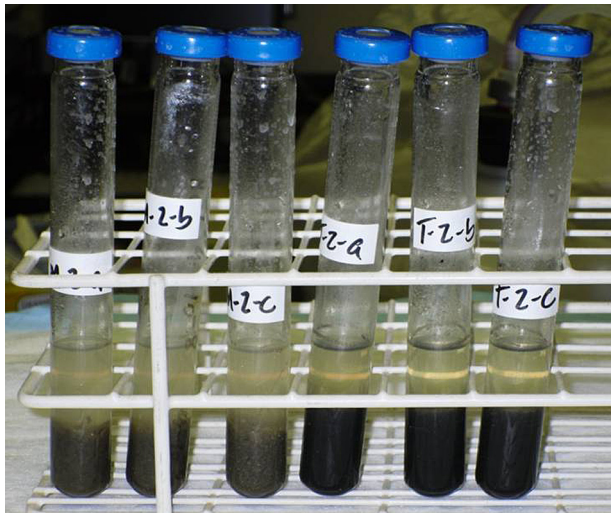

FIGURE 26.7 Sealed test tubes utilized as anaerobic digestor microcosms. Photo courtesy Syreeta Miles, the University of Arizona.

TABLE 26.4 Influence of Wastewater Treatment on Infectious Prion Inactivation

Treatment	Incubation Period	Decrease in Infectious Prions
Mesophilic anaerobic digestion	21 days	4.2 $\log_{10}$
Thermophilic anaerobic digestion	21 days	4.7 $\log_{10}$
Lime treatment of Class B biosolids	2 hours	2.9 $\log_{10}$

Adapted from Miles et al. (2013).

TABLE 26.5 Pathogens and Levels (Geometric Mean) Commonly Found in Waste Residuals

	Bovine	Poultry	Swine	Raw sludge	Class B biosolids
	CFU, PFU, MPN g^{-1}				
Campylobacter jejuni	≈150	≈340	≈460	≈3100	≈2.0
E. coli	≈170				
Listeria monocytogenes	≈600	≈180	≈210	≈2400	≈25
Salmonella	≈630	≈60	≈50	≈2400	≈25
Adenovirus				≈130	≈40
Enterovirus				≈40	≈4.0
Norovirus				≈2.7×10^5	≈1700
Cryptosporidium spp.	≈7.0			≈30	0.7

Table modified from Brooks et al. (2012a).

temperatures. In addition, lime treatment of Class B biosolids was shown to be particularly effective in inactivating infectious prions. Overall, the data suggest that prions do not survive wastewater treatment, and that land application of biosolids is not a viable route of human or animal exposure to prions.

26.4 PATHOGENS OF CONCERN IN ORGANIC RESIDUALS

Pathogenic bacteria and protozoa are known to reside within both Class B biosolids and animal manures. Pathogenic viruses can also be found in biosolids, but not in animal manures. Note also that by definition, Class A biosolids do not contain detectable pathogens. Pathogens routinely associated with either organic residual are shown in Table 26.5.

Whereas human pathogenic viruses are found exclusively in Class B biosolids, concentrations of the bacterial pathogens are normally found in higher concentrations in animal manures than in biosolids, most likely due to the fact that manures do not undergo treatment (Brooks *et al.*, 2012a).

Manure is known to contain a wide and varied array of bacterial and parasitic pathogens, and depending on its origin, can be a source of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, *Cryptosporidium parvum* and *Giardia lamblia* (Guan and Holley, 2003; Hutchison *et al.*, 2004; McLaughlin *et al.*, 2009). Two issues associated with pathogens found in residuals are regrowth and reactivation.

26.4.1 Regrowth and Reactivation of Pathogens within Organic Residuals

26.4.1.1 Class B Biosolids

Regrowth and reactivation have both been documented as occurring in biosolids, but the two terms are not synonymous (Chen *et al.*, 2011). Reactivation is defined as a large increase in fecal coliform or *E. coli* in biosolids collected immediately after centrifugation or other dewatering processes, when compared with the feed into the dewatering equipment (WERF, 2006). Regrowth refers to an additional increase in the density of fecal indicators or *E. coli* upon storage of the biosolids over a period of hours or days.

Reactivation is of concern since studies have documented a large increase in fecal coliforms of several

orders of magnitude in a short period of time that would preclude increases due to normal growth that could occur due to binary fission. The phenomenon was first observed by Donald Hendrickson in 2001 (Hendrickson *et al.*, 2004). Because it could not rationally be explained, reactivation was immediately controversial, and had implications for the designation of biosolids as Class A or B. This resulted in numerous studies on the process of reactivation, which indicated that reactivation did occur following dewatering by centrifugation, but not following dewatering with the use of a belt filter press (Erdal *et al.*, 2003). Different hypotheses have been developed to explain the phenomenon of reactivation (Information Box 26.4).

Many studies have also evaluated the potential for growth and/or regrowth of indicators and pathogens in land amended biosolids with either Class A or B material. These studies have resulted in a number of terms being coined to explain the increase in numbers (Information Box 26.5). Studies evaluating the growth and/or regrowth of *Salmonella* and fecal indicators have produced mixed results, some showing increased numbers following land application, and some showing no such increase in numbers. This is most likely due to the different ways in which studies have been conducted, including laboratory studies versus field studies. In addition, some studies have monitored numbers of organisms that survived wastewater treatment and are subsequently introduced into soil via biosolids, as compared to other studies where laboratory strains of organisms have been inoculated into biosolids and the numbers monitored (Zaleski *et al.*, 2005b). Normally, it is a thought that monitoring the organisms that survive treatment is the preferred option, with field studies being more "real world" than laboratory studies. Whereas the growth and regrowth of fecal indicators in biosolid amended soil has frequently been noted, corresponding studies showing growth of *Salmonella* are far less frequent (Zaleski *et al.*, 2005b). Also, regrowth of fecal indicators is frequently associated with increased moisture following rainfall events (Pepper *et al.*, 1993).

Regrowth of *Salmonella* in Class A biosolids was observed after rainfall produced saturated conditions (Zaleski *et al.*, 2005a). Subsequently, this was shown to be recolonization following contamination with bird feces, since the *Salmonella* serotypes identified prior to the increase in numbers were different from those identified after the rainfall event. This has implications for the storage of Class A biosolids, which should be covered during storage for two reasons: (1) to prevent saturated conditions during rainfall events; and (2) to prevent recolonization by bird or animal feces.

Information Box 26.4 Main Hypotheses Developed to Explain Reactivation

- Clumping of bacteria when the biosolids were originally assayed, followed by desegregation of clumps into single cells following dewatering
- Formation of viable but nonculturable bacteria (VBNC) during wastewater treatment, and subsequent reactivation of the VBNC due to a signaling substance released into the centrate during centrifugation (WERF, 2006)

To date, the VBNC hypothesis is the most likely explanation for reactivation. Use of quantitative polymerase chain reaction (qPCR) to enumerate *E. coli* showed that copy numbers were not significantly different before and after dewatering, which supports the VBNC concept (Higgins *et al.*, 2007). Reactivation not only potentially affects the designation of biosolids as Class A or B, but also raises the possibility of reactivation of pathogens. Increased numbers of fecal coliforms in Class A biosolids has also been reported (Jolis, 2006).

Information Box 26.5 Terms and Definitions Utilized for Increased Numbers of Pathogens and Indicators

Growth:	Increase in detectable numbers of a known microbial population over time.
Regrowth:	Increase in numbers after a period of decline in numbers.
Recolonization:	Reintroduction of bacteria into biosolids followed by growth.
Reactivation:	Large rapid increase in numbers that cannot be ascribed to growth by binary fission.

26.4.1.2 Manure

Very few studies have demonstrated the regrowth of either indicator or pathogenic bacteria in manure. Composted manure has been demonstrated to support regrowth of *E. coli* O157:H7, particularly in compost with high moisture levels, above 30%, and low background bacterial counts. In other instances, regrowth of enterococci and *E. coli* have been demonstrated in poultry litter applied land following simulated rainfall (Brooks *et al.*, 2009, 2012b), and cow pats in fields (Sinton *et al.*, 2007). As in the situations with compost and biosolids, the driving factor behind regrowth was the presence of readily available organic nutrients and substrates and moisture. Finally, note that when pathogens are introduced into soil, some may adapt and be capable of survival within the soil, but only at the cost of the loss of pathogenicity (Ishii *et al.*, 2006). Similarly, *E. coli* has been shown to lose virulence during manure storage (Duriez *et al.*, 2008).

However, regardless of how low the incidence of infections from pathogens in soil is, people want to know how likely it is that *they* will get infected. To answer

this question we can utilize the process of quantitative microbial risk assessment.

26.5 QUANTITATIVE MICROBIAL RISK ASSESSMENT OF PATHOGENS IN ORGANIC RESIDUALS

Quantitative microbial risk assessment (QMRA) can be used to compare pathogen risks from manure to municipal biosolids. Based on recent and historical data, pathogen levels can be estimated for a variety of manure sources as well as for Class B biosolids. Following the risk paradigm (see Chapter 24) the risk for a specific exposure scenario can be determined, utilizing the data from Table 26.4 and transport and inactivation decay models. A comprehensive study recently determined the risks inherent from various exposures to bovine, swine and poultry manures, raw sludge and Class B biosolids (Information Box 26.6). These comparisons highlighted the importance of waste treatment, time and dilution in attenuating pathogen levels. When risks from biosolids and manure were directly compared for *Campylobacter*, *Cryptosporidium*, *Listeria* and *Salmonella*, manure invariably had greater risks under any of the exposure scenarios (e.g., fomites, fresh food crops, aerosol). However, biosolids pose an additional risk due to the incidence of human viruses within biosolids. The differences in risk can be explained by a large number of bacterial pathogens in manure with low infectivity, while biosolids risk can be attributed to a low level of highly infectious viral pathogens. The study also demonstrated the conservative nature of the EPA Part 503 rule, which dictates long delays between land application and fresh food crop harvest. Overall, a 4-month delay was more than adequate to reduce risk for nearly all microbial pathogens' acceptable levels, except viral pathogens and *Cryptosporidium*. Occupational risk can also be considered from exposures inherent to the land application and handling of waste residuals. Public

Information Box 26.6 Application of Risk Paradigm to Pathogens in Land Applied Manures and Biosolids

QMRA approaches can be applied to various manure or biosolids land application scenarios including: fomite contact; soil ingestion (both intentional and accidental); crop ingestion; runoff contamination; and aerosol exposures. Modeling these scenarios can be difficult, often requiring knowledge of pathogen survival in soil amended with residuals, or runoff water transport characteristics. As an example of calculating predicted risk, the following risk paradigm is applied to fomite and fresh food crops contaminated with pathogens from biosolids or manures.

1. Define *Salmonella* level (i.e., define hazard and hazard level)—for this simulation, we assume *Salmonella* at 160 and 5 CFU g^{-1} in bovine manure and Class B biosolids, respectively.
2. Define the route of exposure; in this case we define exposure via: (a) fomite contact; and (b) fresh food crop ingestion. For this simulation we can refer to the exposure models established by Brooks *et al.* (2012a):
   a. Fomite contamination (*fc*),
      $fc = rc \times ft$;
      where:
      rc = residual pathogen level per g defined in step 1,
      ft = the amount of residual transferred to the fomite, 0.1 g, and
      fc = the pathogen level on the fomite. We will assume no decay or decrease in pathogen concentration.
   b. Fresh food crop contamination (cc),
      $cc = rc \times dr \times (1/10^{sr}) \times rr \times wr \times 1000$;
      where:
      dr = soil dilution rate of 1.75×10^{-3}
      sr = pathogen decay rate in soil, equivalent to 0.422 $\log_{10}$ 7 d^{-1}
      rr = the percentage of soil particles remaining on a crop following harvest, equivalent to 0.02 or 2%
      wr = the percentage of soil particles remaining on a crop following washing, equivalent to 0.10 or 10%, and
      cc = the crop pathogen level per kg.
3. Model the dose–response using dose exposures and dose response models:
   a. Dose exposure (d), $d = ec \times ds$;
      where:
      ec = pathogen level in each separate exposure scenario define in 2(a) or 2(b), fc and cc, respectively, and
      ds = the dose amount.
      i. In the fomite situation, a fomite to hand (43%) and hand to mouth (36%) transfer rate are used, which modify the previous equation to $d = ec \times (0.43 \times 0.36)$.
      ii. ds for the food crop scenario is equivalent to 0.292 kg for a 68 kg adult. We will assume the 0.292 kg dose as a one-time exposure.
   b. The beta–Poisson dose–response model for *Salmonella* consists of $P_i = 1 - [1 + (d/N_{50}) \times (2^{1/\alpha} - 1)]^{-\alpha}$;
      where:
      d = the dose exposure from step 3a
      $N_{50} = 2.4 \times 10^4$, $\alpha = 3.1 \times 10^{-1}$, and
      P_i is the probability of infection
4. The risk is then calculated and characterized.
   a. P_i for *fomite* exposures to *Salmonella* = 3×10^{-4} chance of infection from exposures to bovine manure on a fomite with no decay.
      Exposure to biosolids contaminated fomites is equivalent to 8×10^{-6}.
   b. The *crop* exposure yielded risks of 9×10^{-6} for bovine exposures, while biosolids exposures yielded risks of 2×10^{-7}.

risks arise from exposures either during land application (e.g., aerosol drift), or following land application (e.g., fresh food crops). Differences in these risks can be substantial as occupational exposures occur when the waste residuals are "fresh" with high concentrations of pathogens, while public exposure occurs following multiple intervening steps such as environmental inactivation, dilution and disinfection steps. The application of QMRA to this field is still new and exciting, and reminds us that much is unknown regarding pathogen behavior in soil, water, and air or on crops, and that many of these interactions are dynamic and unpredictable.

QUESTIONS AND PROBLEMS

1. Define the differences between sewage, sewage sludge, Class A biosolids and Class B biosolids.
2. What are the major hazard differences between land application of Class B biosolids and cow manure?
3. Discuss the difference in the potential for infections from land application of Class B biosolids versus animal manures.
4. Which gives the greatest risk of infection during land application of organic residuals: direct contact with the land applied residual, or indirect effects to communities due to offsite transport of pathogens as aerosols or via runoff to crops?
5. Using the equations located in Information Box 24.6, calculate the risk of infection for a *Salmonella* level of 3000 CFU g^{-1} and a soil pathogen decay rate (sr) of 1.422 $\log_{10}$ 7 d^{-1} on a fresh food crop contaminated with poultry manure. Assume 7 days of decay, a soil dilution rate of 1.75×10^{-3}, and 2 and 10% of soil particles remaining on crops following harvest and washing, respectively. Assume a 68 kg adult and a one-time exposure.
6. How could you increase the pathogen level on a fomite or crop without increasing the starting pathogen level? What type of scenario(s) would increase the pathogen level in residual waste? Is pathogen level (rc) the most important variable in these QMRAs? Why or why not?
7. Would wearing gloves, in the fomite simulation, always reduce risk?

REFERENCES AND RECOMMENDED READING

Brooks, J. P., Tanner, B. D., Gerba, C. P., Haas, C. N., and Pepper, I. L. (2005a) Estimation of bioaerosol risk of infection to residents adjacent to a land applied biosolids site using an empirically derived transport model. *J. Appl. Microbiol.* **98**, 397–405.

Brooks, J. P., Tanner, B. D., Josephson, K. L., Haas, C. N., Gerba, C. P., and Pepper, I. L. (2005b) A national study on the residential impact of biological aerosols from the land application of biosolids. *J. Appl. Microbiol.* **99**, 310–322.

Brooks, J. P., Tanner, B. D., Gerba, C. P., and Pepper, I. L. (2006) The measurement of aerosolized endotoxin from land application of Class Biosolids in Southeast Arizona. *Can. J. Microbiol.* **52**, 150–156.

Brooks, J. P., Rusin, P. A., Maxwell, S. L., Rensing, C., Gerba, C. P., and Pepper, I. L. (2007) Occurrence of antibiotic-resistant bacteria and endotoxin associated with the land application of biosolids. *Can. J. Microbiol.* **53**, 1–7.

Brooks, J. P., and McLaughlin, M. R. (2009) Antibiotic resistant bacterial profiles of anaerobic swine lagoon effluent. *J. Environ. Qual.* **38**, 2431–2437.

Brooks, J. P., Adeli, A., Read, J. J., and McLaughlin, M. R. (2009) Rainfall simulation in greenhouse microcosms to assess bacterial-associated runoff from land-applied poultry litter. *J. Environ. Qual.* **38**, 218–229.

Brooks, J. P., McLaughlin, M. R., Scheffler, B., and Miles, D. M. (2010) Microbial and antibiotic resistant constituents associated with biological poultry litter within a commercial poultry house. *Sci. Total Environ.* **408**, 4770–4777.

Brooks, J. P., Brown, S., Gerba, C. P., King, G. M., O'Connor, G. A., and Pepper, I. L. (2011) Land application of organic residuals: Public health threat or environmental benefit? American Society of Microbiologists. Washington, DC.

Brooks, J. P., McLaughlin, M. R., Gerba, C. P., and Pepper, I. L. (2012a) Land application of manure and Class B biosolids: an occupational and public quantitative microbial risk assessment. *J. Environ. Qual.* **41**, 2009–2023.

Brooks, J. P., McLaughlin, M. R., Adeli, A., and Miles, D. M. (2012b) Runoff release of fecal bacterial indicators as influenced by two poultry manure application rates and $AlCl_3$ treatment. *J. Water Health* **10**, 619–628.

Burkholder, J., Libra, B., Weyer, P., Heathcote, S., Kolpin, D., Thorne, P. S., and Wichman, M. (2007) Impacts of waste from concentrated animal feeding operations on water quality. *Environ. Health Perspect.* **115**, 308–312.

Castellan, R. M., Olenchock, S. A., Kinsley, K. B., and Hankinson, J. L. (1987) Inhaled endotoxin and decreased spirometric values: an exposure-response relation for cotton dust. *New Engl. J. Med* **317**, 605–610.

Chee-Sanford, J. C., Mackie, R. I., Krapac, I. G., Lin, Y. F., Yannarell, A. C., Maxwell, S., and Aminov, R. I. (2009) Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *J. Environ. Qual.* **38**, 1086–1108.

Chen, Y. C., Murthy, S. N., Hendrickson, D., Araujo, G., and Higgins, M. J. (2011) The effect of digestion and dewatering on sudden increases and regrowth of indicator bacteria after dewatering. *Wat. Environ. Res.* **83**, 773–783.

Chetochine, A., Brusseau, M. L., Gerba, C. P., and Pepper, I. L. (2006) Leaching of phage from Class B biosolids and potential transport through soil. *Appl. Environ. Microbiol.* **72**, 665–671.

Chinivasagam, H. N., Tran, T., Maddock, L., Gale, A., and Blackall, P. J. (2009) Mechanically ventilated broiler sheds: a possible source of aerosolized *Salmonella, Campylobacter*, and *Escherichia coli*. *Appl. Environ. Microbiol.* **75**, 7417–7425.

Clark, C. S., Rylander, R., and Larsson, L. (1983) Levels of gram-negative bacteria, *Aspergillus fumigatus*, dust and endotoxin at compost plants. *Appl. Environ. Microbiol.* **45**, 1501–1505.

Donham, K. J., Cumro, D., Reynolds, S. J., and Merchant, J. A. (2000) Dose-response relationships between occupational aerosol exposures

and cross-shift declines of lung function in poultry workers: recommendations for exposure limits. *J. Occup. Environ. Med.* **42**, 260–269.

Duchaine, C., Thorne, P. S., Merizux, A., Grimard, Y., Whitten, P., and Cormier, Y. (2001) Comparison of endotoxin exposure assessment by bioaerosol impinger and filter-sampling methods. *Appl. Environ. Microbiol.* **67**, 2775–2780.

Dungan, R. S. (2010) Fate and transport of bioaerosols associated with livestock operations and manures. *J. Anim. Sci* **88**, 3693–3706.

Dungan, R. S., and Leytem, A. B. (2009) Airborne endotoxin concentrations at a large open-lot dairy in Southern Idaho. *J. Environ. Qual.* **38**, 1919–1923.

Duriez, P., Zhang, Y., Lu, Z., Scott, A., and Top, E. (2008) Loss of virulence genes in *Escherichia coli* populations during manure storage on a commercial swine farm. *Appl. Environ. Microbiol.* **74**, 3935–3942.

Erdal, Z. K., Mendenhall, T. C., Neely, S. K., Wagoner, D. L., and Quigly, C. (2003) Implementing improvements in a North Caroliner Residuals Management Program. Proc. WEF/AWWA/CWEA Joint Residuals and Biosolids Management Conference; Baltimore, MD.

Guan, T. Y., and Holley, R. A. (2003) Pathogen survival in swine manure environments and transmission of human enteric illness. A review. *J. Environ. Qual.* **32**, 383–392.

Hendrickson, D., Denard, D., Farrell, J., Higgins, M., and Murthy, S. (2004) Reactivation of fecal coliforms after anaerobic digestion and dewatering. Proc. Water Environment Federation Annual Biosolids and Residuals Conference; Salt Lake City, Utah. Water Environment Federation: Alexandria, VA.

Higgins, M. J., Chen, Y. C., Murthy, S. N., Hendrickson, D., Farrell, J., and Shafer, P. (2007) Reactivation and growth of non-culturable *E. coli* in anaerobically digested biosolids after dewatering. *Wat. Res.* **44**, 665–673.

Hinckley, G. T., Johnson, C. J., Jacobson, K. H., Bartholomay, C., McMahon, K. D., McKenzie, D., *et al*. (2008) Persistence of pathogenic prion protein during simulated wastewater treatment processes. *Environ. Sci. Technol.* **42**, 5254–5259.

Hinesly, T. D., Jones, R. L., and Ziegler, E. L. (1972) Effects on corn by application of heated anaerobically digested sludge. *Comp. Sci.* **13**, 26–30.

Hutchison, M. L., Walters, L. D., Avery, S. M., Synge, B. A., and Moore, A. (2004) Levels of zoonotic agents in British livestock manures. *Lett. Appl. Microbiol.* **39**, 207–214.

Ishii, S., Ksoll, W. B., Hicks, R. E., and Sadowski, M. J. (2006) Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Appl. Environ. Microbiol.* **72**, 612–621.

IWMI (International Water Management Institute) (2010) In "Wastewater irrigation and health: assessing and mitigating risk in low-income countries" (P. Dreschel, C. A. Scott, L. Raschid-Sally, M. Redwood, and A. Bahri, eds.), Earthscan, London/Sterling, VA.

Jolis, D. (2006) Regrowth of fecal coliforms in Class A biosolids. *Wat. Environ. Res.* **78**, 442–445.

Kirchmayr, R., Reichl, H. E., Schildorfer, H., Braun, R., and Somerville, R. A. (2006) Prion protein: detection in "spiked" anaerobic sludge and degradation experiments under anaerobic conditions. *Wat. Sci. Technol.* **53**, 91–98.

Marshall, B. M., and Levy, S. B. (2011) Food animals and antimicrobials: impacts on human health. *Clin. Microbiol. Rev.* **24**, 718–733.

McLaughlin, M. R., Brooks, J. P., and Adeli, A. (2009) Characterization of selected nutrients and bacteria from anaerobic swine manure lagoons on sow, nursery, and finisher farms in the Mid-South USA. *J. Environ. Qual.* **38**, 2422–2430.

Michel, O. (2003) Role of lipopolysaccaride (LPS) in asthma and other pulmonary conditions. *J. Endotoxin Res.* **9**, 293–300.

Miles, S. L., Sun, W., Field, J. A., Gerba, C. P., and Pepper, I. L. (2013) Survival of infectious prions during anaerobic digestion of municipal sewage sludge and lime stabilization of Class B biosolids. *J. Res. Sci. Technol.* **10**, 69–75.

Miles, S. L., Takizawa, K., Pepper, I. L., and Gerba, C. P. (2011) Survival of infectious prions in water. *J. Environ. Sci. Health* **A46**, 938–943.

NEBRA (North East Biosolids and Residuals Association) (2007) A National Biosolids Regulation, Quality, End Use, and Disposal Survey. Final Report, July 20, 2007.

Neilson, J. W., Josephson, K. L., Pepper, I. L., Arnold, R. B., DiGiovanni, G. D., and Sinclair, N. A. (1994) Frequency of horizontal gene transfer of a large catabolic plasmid (pJP4) in soil. *Appl. Environ. Microbiol.* **60**, 4053–4058.

Nichols, T. A., Pulford, B., Wyckoff, A. C., Meyerett, C., Michel, B., Gertig, K., *et al*. (2009) Detection of protease-resistant cervid prion protein in water from a CWD-endemic area. *Prion* **3**, 171–183.

NRC (National Research Council) (2002) "Biosolids Applied to Land: Advancing Standards and Practices," National Academy Press, Washington, DC.

Olenchock, S. A. (2001) Airborne endotoxin. In "Manual of Environmental Microbiology" (C. J. Hurst, R. L. Crawford, G. R. Knudsen, M. J. McInerney, and L. D. Stetzenbach, eds.), 2nd ed., ASM Press, Washington, DC, pp. 814–826.

Organic Trade Association (2012) Manure Facts. Washington, DC.

Pepper, I. L., Josephson, K. L., Bailey, R. L., Burr, M. D., and Gerba, C. P. (1993) Survival of indicator organisms in Sonoran desert soil amended with sewage sludge. *J. Environ. Sci. Health* **A28(6)**, 1287–1302.

Prusiner, S. B. (ed.) (2004) "Prion Biology and Diseases," Cold Spring Harbor Laboratory Press, Woodbury, New York.

Rusin, P., Maxwell, S., Brooks, J., Gerba, C., and Pepper, I. (2003) Evidence for the absence of *Staphylococcus aureus* in land applied biosolids. *Environ. Sci. Technol.* **37**, 4027–4030.

Sharif El, N., Douwes, J., Hoe, P. H. M., Doekes, G., and Nemery, B. (2004) Concentrations of domestic mite and pet allergens and endotoxin in Palestine. *Allergy* **59**, 623–631.

Sinton, L. W., Braithwaite, R. R., Hall, C. H., and Mackenzie, M. L. (2007) Survival of indicator and pathogenic bacteria in bovine feces on pasture. *Appl. Environ. Microbiol.* **73**, 7917–17925.

Tanner, B. D., Brooks, J. P., Haas, C. N., Gerba, C. P., and Pepper, I. L. (2005) Bioaerosol emission rate and plume characteristics during land application of liquid Class B biosolids. *Environ. Sci. Technol.* **39**, 1584–1590.

Taylor, D. M. (2000) Inactivation of transmissible degenerative encephalopathy agents: a review. *Vet. J.* **159**, 10–17.

U.S. EPA (U.S. Environmental Protection Agency) (1993) The standards for the use or disposal of sewage sludge. In "Final 40 CFR Part 503 Rules. EPA 822/Z–93/001," U.S. Environmental Protection Agency.

U.S. EPA (U.S. Environmental Protection Agency) (2000) A guide to field storage of biosolids and the organic by-products used in agriculture and for soil resource management. EPA/832–B–00–007, Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency) (2004) Risk assessment evaluation for concentrated animal feeding operations. EPA 600/R−04/042.

Water Environment Federation (WERF) (2006) Reactivation and regrowth of fecal coliforms in anaerobically digested biosolids. Technical Practice Update Report: Alexandria, VA.

Zaleski, K. J., Josephson, K. L., Gerba, C. P., and Pepper, I. L. (2005a) Potential regrowth and recolonization of *Salmonella* and indicators in biosolids and biosolids amended soil. *Appl. Environ. Microbiol.* **71**, 3701−3708.

Zaleski, K. J., Josephson, K. L., Gerba, C. P., and Pepper, I. L. (2005b) Survival, growth, and regrowth of enteric indicator and pathogenic bacteria in biosolids, compost, soil, and land applied biosolids. *J. Residuals Sci. Technol.* **2**, 49−63.