

Survival, Growth, and Regrowth of Enteric Indicator and Pathogenic Bacteria in Biosolids, Compost, Soil, and Land Applied Biosolids

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ABSTRACT: In the US approximately 60% of all biosolids are currently land applied. Although it is known that bacteria in biosolids normally decrease to low or non-detectable levels following treatment, a major concern is that regrowth of pathogens may occur. Specifically the question arises: "Does regrowth occur following reintroduction or recolonization of pathogens after land application or during storage under favorable conditions." The following paper reviews available information on survival and potential regrowth of pathogenic and indicator bacteria in biosolids, compost, soil, and land applied biosolids. Based on the literature, a conceptual framework is provided to explain the phenomenon of "regrowth."

INTRODUCTION

MILLIONS of dry tons of biosolids are produced every year. Recycling of them and the safety of disposal is an issue at the center of a national debate. Due to the nutrient content of biosolids, they can be reused beneficially as a soil amendment on numerous areas, such as parks, forests, and agricultural land [41]. Biosolids can also be sold in bulk to consumers for use on home gardens or lawns, as in the case of Milorganite®.

One of the reasons for the continuing debate regarding land application is that even though biosolids treatment is required, metals, harmful toxins, and pathogens may still be present following treatment. Microorganisms present in biosolids may include, viruses, bacteria, helminthes, and protozoa [11,12,30]. Many concerns and allegations exist regarding the effect of land applied biosolids on the health of the general population and personnel in the wastewater industry [6,18,20,24]. However, there has never been an established link between land application and illness.

Although the United States Environmental Protection Agency supports and regulates the land application of biosolids, the concerns of pathogens affecting humans and animals through direct contact, transfer to

groundwater, run-off into surface water, transfer to food crops grown on land applied field, or even through aerosols remain [40,42]. Although it is known that bacteria in biosolids normally decrease to low or non-detectable levels following treatment, a major concern is that regrowth of pathogens may occur. Specifically the question arises: "Does regrowth occur following reintroduction or recolonization of pathogens after land application or during storage under favorable conditions."

SALMONELLA AND INDICATORS IN BIOSOLIDS

The growth of bacterial pathogens during the storage of biosolids prior to land application is of concern. Gibbs et al. tested anaerobically digested dewatered sludge in 1m high piles placed on a sandy soil over a limestone base [13]. Two studies were conducted, including a 60 week study from autumn to winter and a 24 week study from summer to winter. Samples were taken from depths of 20–40 cm and 40–60 cm below the surface of the piles and were assayed for fecal coliforms, fecal streptococci, and *Salmonella*.

In the first trial, fecal streptococci decreased but always remained detectable throughout the experiment. Fecal coliforms were undetected after 34 weeks, while *Salmonella* were undetected after 16 weeks. Following the commencement of the winter rains, fecal coliforms

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increased to levels of 1.2×10^6 CFU/gram, while *Salmonella* levels were detected at 2.2 MPN/gram. For the second trial, an increase of fecal coliforms and *Salmonella* occurred corresponding to rainfall events.

For both trials, rainfall did not affect the moisture content of the piles as they both remained around 80% throughout both studies. From these experiments there seemed to be no definite explanation for the regrowth seen in the sludge piles, as moisture was not a factor. It was concluded that fecal contamination from animals was not the reason for the observed regrowth because the *Salmonella* serotypes detected in the biosolids throughout the study were all found to be *Salmonella mbandaka*. Rainfall may have been important for increasing a necessary substrate for growth to certain areas of the stored biosolids. This could be possible as Burge et al. were able to extract a substrate from biosolids that was able to support growth of *Salmonella*, and could potentially permit regrowth [4]. From this study it was concluded that storage of biosolids for 1 year is an ineffective means for treating biosolids due to the potential for repopulation of fecal bacteria.

Ahmed and Sorensen evaluated pathogen inactivation during storage of biosolids [1]. The pathogens tested were *S. typhimurium*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Ascaris suum*, bacteriophage f2, and poliovirus. The biosolids tested were aerobically digested, and two of the samples collected had been stored through winter and had undergone freezing and thawing, whereas the third sample was produced after the winter period. Samples seeded with the pathogens were incubated under both anaerobic and aerobic conditions in reactors at 5, 22, 38, and 49.5°C for up to 62 days. There was no difference in the death of the organisms under aerobic or anaerobic conditions. Pathogen destruction in stored biosolids occurred at all temperatures, and survival decreased as the temperature increased. Also, more rapid destruction of *Salmonella* occurred in two of the tests where the moisture contents were lower at 47.7% and 50%, compared to 67.3% moisture.

Research by Ward et al. determined the response of *Klebsiella*, *Enterobacter*, *Proteus mirabilis*, *Salmonella typhimurium*, *Escherichia coli*, and *Streptococcus faecalis* to moisture loss through evaporation at 21°C in the laboratory [45]. In raw sterilized sludge with 5% solids, seeded enteric bacteria initially increased in concentration with dewatering. This increase was always followed by a consistent decrease in numbers with further dewatering especially below 50%

moisture. A 1–2 log reduction was seen for all microorganisms except *Proteus*, which showed a 4 log reduction within 7 days as biosolids percent solids increased to 95%. For indigenous bacteria in non-sterilized raw sludge, the same basic pattern of initial growth then subsequent inactivation was observed, except more growth occurred during the initial dewatering.

In an accompanying paper, Yeager and Ward determined the effects of moisture content on survival and potential regrowth of bacteria during long-term storage of biosolids [47]. According to the authors, bacterial regrowth would be directly related to the moisture content due to the necessity of water for bacterial growth and survival. Sterilized raw sludge samples dried by natural evaporation at 21°C were brought to different moistures ranging from 10–95% solids, and were seeded with *S. faecalis*, *P. mirabilis*, or *S. typhimurium*. When the samples were tightly sealed and stored at 37°C, growth was seen for every organism in samples containing $\leq 75\%$ solids, while no growth was seen in samples containing $\geq 85\%$ solids.

The results of this study led to the suggestion that sand-bed drying of sludge and water loss may not adequately inactivate bacteria in sludge, however these results may not be supported under field conditions [45,47]. According to the authors, long term storage of sludge with moisture contents maintained between 10 and 50% may be an effective way to inactivate bacterial pathogens, while storage at less than 10% moisture may support long-term survival of *Salmonella*. This is in comparison to Gibbs et al. who stated that storage is an ineffective means for inactivation of bacterial pathogens [13].

Various studies were conducted in our laboratory to determine the survival and regrowth potential of indigenous indicator microorganisms and *Salmonella* in biosolids [48]. Laboratory studies were performed in order to determine the survival of coliforms and *E. coli* in anaerobically digested biosolids under conditions of constant temperature and moisture and constant temperature with variable moisture. When desiccation was prevented, both organisms decreased over time even though water was not being lost. Survival decreased as the temperatures tested increased from 45°C to 57.5°C. When biosolids were allowed to dry out, increased temperature and rates of desiccation decreased survival for both coliforms and *E. coli*. Survival and potential regrowth of *Salmonella* and fecal coliforms were then determined during field studies involving the use of solar drying beds. In both anaerobically digested and aer-

obically digested biosolids, both organisms decreased as the temperature and rate of desiccation increased. Regrowth for both *Salmonella* and fecal coliforms was observed following rainfall. It was determined that the high levels of organisms present in the biosolids after regrowth was most likely due to fecal contamination from birds.

Summary

1. Regrowth of indicators and *Salmonella* is possible in biosolids under conditions of favorable moisture and temperature, or substrate availability.
2. Indigenous microorganisms present in biosolids are important for controlling potential regrowth of bacterial pathogens.
3. Storage of biosolids may be effective in reducing pathogen levels if biosolids are maintained at specific controlled moisture and temperature conditions.

SALMONELLA AND INDICATORS IN COMPOST

Composting is also an effective means for reducing pathogens, but there is a concern for the possibility of regrowth due to outside contamination during storage. Experiments by Sidhu et al. were performed with anaerobically digested dewatered biosolids that were subsequently composted using windrows [34]. The compost ranged in age from two weeks to two years. The main objectives were to determine the role of indigenous microflora and maturity in the inhibition of *Salmonella* regrowth. The work consisted of two separate studies using sterile and non-sterile samples seeded with *Salmonella*. A rifampicin-resistant strain of *S. typhimurium*, shown to have a higher maximum growth rate compared to other strains of *Salmonella* and *E. coli*, was used for all experiments.

In the compost sterilized by autoclaving, *Salmonella* grew rapidly within 30 hours of inoculation and incubation. Seeded *Salmonella* grew to levels of 10^8 /gram in compost stored for 2 weeks, and although growth occurred in compost stored for 33 and 117 weeks the concentrations and growth rate decreased. The die-off rates were low in sterilized biosolids, whereas limited growth and increased die-off rates occurred in non-sterilized samples seeded with *Salmonella*. It was found that *Salmonella* growth increased significantly when indigenous microorganisms were not present,

and the presence of indigenous microflora seemed to be the most important factor suppressing *Salmonella* growth. The authors speculated that the presence of somatic *Salmonella* phage, the ability of some indigenous microorganisms to outcompete *Salmonella* for limited nutrients, or the higher activity of indigenous microorganisms during favorable conditions may be the reason for suppression of growth. The conclusions from this study were that all composts seem to have the potential for regrowth, and the long-term storage of the compost is not recommended. The inactivation rate of *Salmonella* decreased significantly with longer storage times, most likely due to the decline in indigenous microflora over time.

Due to the possibility of the introduction or contamination of *Salmonella* into compost by vectors, the potential for regrowth of inoculated *Salmonella* in compost was evaluated by Hussong et al. [15]. Thirty one samples composted by the Beltsville aerated pile method were inoculated with *Salmonella*. Irradiated and non-irradiated samples were both tested for growth of *Salmonella* after incubation at 37°C. *Salmonella* was shown to grow well in all but two of the irradiated composts tested. In the non-irradiated compost, *Salmonella* was not detected in half of the samples within 7 days. Samples were also incubated for a period of 6 months, and in non-irradiated compost, *Salmonella* decreased 2 logs in the first month and was subsequently undetectable. Dried composts with 10, 6, and 7% moisture were also monitored for 26 weeks, and *Salmonella* was shown to decline several logs within 4 weeks in both irradiated and non-irradiated samples. In moist soils, *Salmonella* grew well only when soils were sterilized. The only difference between the irradiated and non-irradiated samples was the presence of other microorganisms, so it was concluded that competition from other organisms suppresses the growth of *Salmonella* in compost. It has been suggested that compost be sterilized as a final treatment, but this could allow for potential regrowth of pathogens due to the lack of competitive indigenous microorganisms in the compost.

Another experiment to assess the role of indigenous microflora in the suppression of bacterial pathogens under different temperature and moisture conditions was performed by Pietronave et al. at the University of Eastern Piedmont in Novara, Italy [28]. Both sterile and non-sterile composts, adjusted to moisture contents of 10, 40, and 80%, were tested. Samples were inoculated with *S. arizonae* or an enteropathogenic strain of *E. coli* and were incubated at either room temperature or 37°C.

In non-sterile compost, with moisture contents of 40 and 80%, *Salmonella* concentrations increased briefly and then declined below the original seeded levels within two weeks. The concentrations of organisms were lower in the 40% moisture sample than in the 80% moisture sample. Temperature did not have an effect at higher moisture contents, but it did have an effect on the survival of *Salmonella* at 10% moisture. After 48 hours, concentrations were much lower in non-sterilized compost incubated at 27°C than in samples incubated at 37°C. In sterilized samples, *Salmonella* concentrations increased within 24 hours for all moisture levels, and then decreased gradually over 30 days. For *E. coli*, low moisture had a detrimental effect on the survival in both sterile and non-sterilized composts. At 40 and 80% moisture, *E. coli* levels initially increased and then decreased below the seeded dose at 40% moisture, while remaining above the seeded dose at 80% moisture. From these experiments it was concluded that indigenous microorganisms in non-sterilized composts suppressed the growth of both *Salmonella* and *E. coli* in all trials. Moisture, more than temperature, played an important role in the regrowth of both *E. coli* and *Salmonella*, and high moisture and increased temperature positively affect regrowth.

Biosolids composted for 3 days at 55°C are thought to be free of pathogens and safe for distribution to consumers. The major concern for compost is the possibility for re-contamination or recolonization of pathogens from outside sources and subsequent regrowth of these organisms to hazardous levels. Research by Millner et al. was performed in order to address this concern [23]. They focused on the importance of indigenous microflora on the growth and suppression of *Salmonella* in composted sewage sludge. Three different environmental isolates of *Salmonella*, along with three laboratory strains, were inoculated into compost along with several isolates of actinomycetes, fungi, and bacteria. The initial tests showed that only 6 fungi isolates inhibited growth of *Salmonella* while none of the actinomycetes or bacterial isolates caused suppression of growth.

Several different studies were then performed in order to determine suppression or growth in sterile and non-sterile compost samples. Recolonization tests with individual organisms were performed using sterile samples of compost. Inhibitory isolates, found in the previous studies, were either inoculated into compost simultaneously or 7 days prior to inoculation of *Salmonella*. From these studies they found that none of the or-

ganisms that caused inhibition on agar plates (fungi), caused the same inhibition in actual sterile compost.

A series of three separate studies were then performed using different mixtures of microbes found in compost. The first series used compost that had been subjected to different temperatures, which represented different communities of organisms. One sample was collected from the 70°C-plus zone of a 21 day compost, the second sample was from the same zone as the first sample but was incubated at 55°C for an additional week. The third sample was taken from a laboratory operated composter that operated at 55°C, and the final sample was compost from a 25–40°C zone of a 60 day pile. The second series inoculated groups of microorganisms obtained by filter fractionation of compost extracts, into sterile composts 7 days before *Salmonella* was inoculated. The final third series used mixtures of non-coliform or coliform gram-negative bacteria, including *E. coli*, *Enterobacter hafniae*, *Enterobacter sp.*, and *Citrobacter freundii*.

It was observed that the growth of *Salmonella* depended on the types and amounts of microbes present in the samples. The first series of tests showed that suppression of *Salmonella* increased with the presence of gram-negative bacteria and high levels of mesophilic actinomycetes and bacteria. The 70°C treated compost used in this study was the first example of a non-sterile compost that did not show suppression of *Salmonella* growth. In this compost, initial amounts of actinomycetes, bacteria, and fungi levels were very low or even absent. From these studies it was also determined that *Salmonella* death would only occur in the presence of the complete microflora that is found in compost, which occurred when the 60 day compost was used.

For the second series of tests, where organisms in different fractions were used, it was found that fungi were relatively insignificant in the death and suppression of *Salmonella*. It appeared gram-negative bacteria in combination with mesophilic actinomycetes was necessary for a negative effect on growth. In the final studies, it was shown that prior growth of coliforms, before inoculation of *Salmonella*, resulted in a decrease of *Salmonella* to 75% less than that of the inoculum. If *Salmonella* was inoculated simultaneously with coliforms or 7 days after noncoliforms, then *Salmonella* numbers increased.

From these studies it was concluded that *Salmonella* regrowth would be negligible in properly cured composts where the presence of coliforms is established.

The presence of coliforms should not be regarded as a health threat because prior colonization by coliforms and gram-negative bacteria significantly decreased *Salmonella* populations upon reinoculation. It was also determined that a single group of indicators cannot be used to assess the *Salmonella* present in a compost at any given time because the death of *Salmonella* was never related to a single group of microbes.

Russ and Yanko indicate that there is a lack of agreement on the regrowth potential in composted biosolids [31]. Some researchers seem to believe that composting is an effective means for reducing *Salmonella* concentrations below detectable and hazardous levels, while others believe that repopulation (regrowth) of *Salmonella* in composting under certain conditions could be hazardous. They refer to studies done by Epstein and Wilson showing that it was possible for inoculated *Salmonella* at levels of 10^3 bacteria/gram of solid to increase to 10^9 bacteria per gram within a few days of incubation [9]. Russ and Yanko conducted experiments in order to determine if controlling variables such as temperature and moisture could eliminate the potential for *Salmonella* regrowth in compost [31]. Compost was dried in the lab for 1 year until total solids was 92.8% and *Salmonella* was undetected, but the compost was not sterilized. These samples were brought up to different moisture contents of 9, 16, and 21% and were incubated at temperatures of 4, 28, 36, and 44°C in a moist incubator to prevent desiccation. The experiment was also repeated under the same conditions but *Salmonella* was inoculated into the composts before incubation, in order to achieve initial high concentrations of *Salmonella*. In the uninoculated samples, indigenous *Salmonella* populations increased by 4 orders of magnitude within 5 days at 28°C and 36°C in samples at 21% moisture. At 28°C, *Salmonella* was still detected after 21 days of incubation. The inoculated *Salmonella* grew to levels of $>3.3 \times 10^7$ MPN/gram within 5 days again at both 28 and 36°C. It was shown that regrowth of *Salmonella* did occur, but population levels peaked after five days with subsequent die off. After 21 days *Salmonella* was undetectable in the majority of samples. The authors support the idea that repopulation of *Salmonella* is a potential health hazard, due to the fact that they were able to demonstrate regrowth of *Salmonella* even in unsterilized conditions with indigenous microbes present.

Brandon et al. also showed growth of *Salmonella* in sterile compost [3]. *Salmonella* was inoculated into sterile compost at 60% solids and growth to hazardous

levels occurred. Within only two days of incubation the levels had increased from 10^3 /gram to 10^9 /gram.

Sidhu et al. studied the survival of a number of *Salmonella* and *E. coli* strains seeded into sterilized compost [33]. The moisture contents used were between 50 and 55%, and the samples were incubated for 5 days at 37°C in a water bath in order to prevent desiccation of the samples. It only took a limited amount of time for inoculated bacteria to adjust to the environment in the sterilized compost, and seeded bacteria grew very rapidly in sterilized compost achieving levels greater than 10^8 organisms per gram after only 30 hours of incubation. It was recognized that it is important that experiments be done with non-sterilized composts in order to determine a more realistic recolonization potential of *Salmonella* and *E. coli* in compost.

According to Skanavis and Yanko, the greatest potential for health hazards in regards to compost is the use of biosolids products in homes [35]. In homes, there would be an increased exposure risk to children due to yard and garden work, and the ingestion of uncooked vegetables that may be grown in the gardens. These studies monitored *Salmonella* and total and fecal coliforms for one year in: (1) compost; (2) the amendment materials added to compost (rice hulls, sawdust, etc); and (3) the final products that can be distributed to homes. For total and fecal coliforms, the highest levels were found in compost-based products compared to the compost and the amendments alone. It was suggested that regrowth of coliforms occurs post-composting due to the introduction of nutrients present in the amendments. *Salmonella* was not detected in either the compost or amendments alone, but were found in all of the products tested. When *Salmonella* was detected, the majority of the samples complied with the 40 CFR Part 503 pathogen regulations [40]. They also found that the greatest number of samples positive for *Salmonella* occurred during April. *Salmonella* was not isolated in samples where fecal coliforms were less than 1×10^3 MPN/gram, but it is not understood whether the *Salmonella* detected in the soil amendment products resulted from contamination or regrowth of indigenous *Salmonella*. Due to the fact that *Salmonella* was detected at low levels in the compost suggests the potential for regrowth under favorable conditions, and post treatment handling may be a potential cause for *Salmonella* regrowth.

Soares et al. studied regrowth after Schumann demonstrated regrowth following the rewetting of compost [32,37]. They performed comprehensive studies to

evaluate regrowth influenced by moisture, nutrients, and indigenous populations [36]. Other studies were done in order to evaluate regrowth potential along with compost stability [5]. In order to evaluate compost quality in regards to pathogens and the regrowth potential, compost collected from 16 composting facilities in Massachusetts during the summer of 1994 were analyzed for total coliforms and *E. coli*. Samples were inoculated with *E. coli* in order to ensure that the microorganisms would be present and samples were adjusted to 50% moisture if necessary. Fifty gram samples were then incubated in a water bath at 37°C. Two of the samples, which had initial moisture levels of 19.6 and 18.4%, showed substantial growth of *E. coli*, whereas other composts did not show a substantial decrease in populations after 5 days of incubation. It was determined that moisture is one of the major factors in determining stability and the potential for recolonization of compost. It was thought that organic matter is not completely degraded during the composting process when the compost becomes too dry and microbial populations cease activity. This could allow for regrowth of indigenous or reinoculated pathogenic microorganisms after rewetting due to the presence of remaining carbon [37].

Summary

1. Growth of inoculated *Salmonella* and *E. coli* is possible in sterile compost.
2. Growth or regrowth of indicators and *Salmonella* is inhibited in non-sterile compost, most likely due to competition or predation from indigenous microorganisms.
3. Moisture is an important factor in the survival and potential regrowth of *Salmonella* and *E. coli* in compost. Temperature and desiccation become more important in decreasing survival at lower moisture contents.

SALMONELLA AND INDICATORS IN SOIL

Van Donsel et al. looked at the survival of inoculated fecal coliforms and fecal streptococci, used as tracer organisms, and indigenous soil microorganisms in outdoor soil plots [44]. Two types of soil and soil plots were studied: (1) a coarse loam soil in a well shaded area; and (2) a dense clay with exposure to direct sunlight. The locations were not subject to human activity

but squirrels and raccoons were occasionally seen in the area. An *E. coli* tracer was inoculated into the soil at a range of 1.2×10^{10} to 2.8×10^{12} , while the *S. faecalis* tracer was inoculated at 3.8×10^{11} to 1.8×10^{12} . While monitoring these organisms, it was seen that during the summer the indicators died off fairly rapidly at both sites, while surviving longer in the shaded environment. The effect of the protected shaded environment was most notable in the summer and autumn when both indicators lasted longer in the shaded site than in the exposed site. The conditions for both of these seasons were warm, dry, clear conditions. During the winter and spring when the weather was cool and wet, with heavy cloud cover and shortened days, the survival of indicators at both sites were fairly similar. There was also a seasonal difference observed in the survival between the two organisms at both sites. During the summer *E. coli* survived longer than *S. faecalis*, whereas *S. faecalis* survived longer than *E. coli* in the winter and spring. Definitive conclusions regarding survival of organisms between the shaded and exposed site may be difficult to reach from this study. Two different types of soil were present at the two sites and so it is possible that the differences in survival were more related to soil type than environmental factors noted in the studies.

As with many other studies, growth of both tracer organisms and indigenous soil coliforms was seen during wet weather. When fecal coliform levels were low, it was possible to see growth of indigenous soil coliforms closely associated with temperature and rainfall. When rainfall was followed by warm weather, a larger increase in the regrowth of total coliforms was seen than when cool weather followed. When the moisture of the soils was maintained above 20%, without the addition of more water from rainfall, the number of total coliforms increased when temperatures remained elevated for a few days. The only time that a decrease in the number of organisms was seen when the moisture content was high was when sub-freezing conditions occurred. The researchers believe that it is unlikely that regrowth was caused by run off from adjacent areas because even just a light rainfall caused an increase in the number of coliforms in the sites. Also, it was believed that animal contamination was not the reason for the growth, because it occurred simultaneously at both sites, and an increase in the tracer organisms was not seen. The authors concluded that there are a number of conditions which might alter survival of organisms in the soil environment such as moisture, pH,

and even repeated freezing and thawing conditions. They also state the importance of the concept that organisms from wild animals might differ in survival from the laboratory stock organisms used.

Parker and Mee studied the survival of fecal coliforms and *S. adelaide* in two coarse sands from Perth, Australia [26]. The two coarse sands tested were Bassendean and Spearwood sand, with the Bassendean sand having a higher percentage of coarse sand (96.9% compared to 76.9%). Fecal coliforms and *Salmonella* were inoculated into the sands at moisture contents of 5% and 23% (saturated), and were sealed and incubated at 15°C. Increased growth of the organisms was seen within the first 16 days of incubation, and fecal coliforms and *Salmonella* both survived for extended periods of time in all treatments. It was also seen that the organisms survived better in the sand containing the higher percentage of coarse sand (Bassendean), and that survival decreased under saturated conditions. In this experiment coarser soils showed better survival, while Mallman and Litsky showed that *E. coli* survived longer in loam and muck soils than in a sandy soil [22].

Along with soil on agricultural or public lands, there is also a concern regarding the survival of fecal bacteria in marine and freshwater sediments that may be present under sewage outfalls. Davies et al. evaluated the survival of fecal coliforms and fecal streptococci in sediments and the effects of predation by protozoa [7]. Cycloheximide was used in order to inhibit protozoa for predation studies. In both marine and freshwater sediments, fecal coliforms and streptococci decreased over time, with the fecal coliforms decreasing more rapidly. In the predation studies it was observed that numbers of fecal coliforms increased slightly in samples containing cycloheximide, and so it was concluded that growth may be possible in sediments in the absence of predators, but that die off would occur in the presence of natural predators.

Van Donsel and Geldreich determined the survival and relationship of *Salmonella* and indicator organisms in mud taken from a creek that received moderate to heavy pollution from sewage [43]. The mud was stored at 20°C, and within 7 days *Salmonella* and fecal coliforms showed identical survival, which was a 90 percent die off. Fecal streptococci and total coliforms showed increased survival and the total coliforms even increased within the first day of storage. Out of the indicators tested, *Salmonella* activity most closely paralleled the activity of the fecal coliforms. The researchers

concluded that fecal coliforms are good indicators of enteric pathogens.

The importance of the relationship between *Salmonella* detection and moisture in the environment was again shown by Thomason et al. [39]. Samples from a forest area and from pools located on the top of a mountain were taken and analyzed for *Salmonella*. The forest area was utilized by warm and cold-blooded animals but had only limited human activity. In contrast, the top of the mountain had less animal activity but increased human activity due to tourism. The importance of moisture for the survival of *Salmonella* and the evidence that even harsh environments can support the survival of *Salmonella* was documented. In the forest samples, *Salmonella* was not detected in any of the dry samples while 10 of the 12 wet samples were positive. Increased isolation of *Salmonella* was also seen in wet samples compared to dry samples from the mountain weather pools. This raised the question of what happens to the *Salmonella* in the pools during the wet and dry periods. Do the *Salmonella* die off during the drying process and then become reintroduced from outside sources, or do the bacteria survive the drying process, possibly in a “viable but nonculturable” state, and regrow when moisture is again present?

If bacteria become undetectable due to lack of culturability, this could pose a condition for regrowth of *Salmonella* or *E. coli* when soils are rewet. Bogosian et al. investigated whether *E. coli* enters the “viable but nonculturable” (VBNC) state, or whether they just become non-viable in sterile and non-sterile soils [2]. Samples of silt loam were incubated in the dark at 4, 27, and 37°C after inoculation with *E. coli* K-12 strain W3110. When *E. coli* was inoculated into non-sterile soil at high initial concentrations, levels rapidly declined at all temperatures tested. In sterilized soil at all temperatures, a decline in *E. coli* concentrations was not seen even after 100 days of incubation. From these studies it was determined that *E. coli* did not enter the VBNC state. Through the use of plate counts, direct counts, and other methods such as PCR, it was determined that *E. coli* was killed by competition or by predation by protozoa. When cells became nonculturable, resuscitation of these cells was not possible which supports the fact that cells die and do not enter a VBNC state.

Kibbey et al. examined the survival of a fecal streptococcus isolated from raw sewage when it is added to soil [16]. Five soil types, including a silt loam, silty clay loam, and a gravelly loam of different moisture con-

tents, ranging from air dried soil to saturated conditions, were incubated with *S. faecalis* at 4, 10, 25, and 27°C. Although reduction times varied between all soils at different temperatures, *S. faecalis* died rapidly under drier conditions at higher temperatures and survived the longest in moist conditions at the cooler temperatures in all samples. The importance of moisture was seen because at any given temperature, the bacteria survived the longest under saturated conditions. They hypothesized that the increased survival of *S. faecalis* under moist, cool conditions could be due to lack of antagonistic activity by the indigenous microflora. Also, lack of moisture caused a decrease in survival for the organisms due to desiccation, but when moisture was not lacking and temperatures increased, the decrease could be due to thermal effects or increased activity of the indigenous microorganisms.

Freeze-thaw experiments were also performed in the previous experiments in order to evaluate the response of *S. faecalis* to numerous freeze-thaw procedures. Pots were frozen and thawed 1, 4, and 8 times, and survival was lowest in the treatment with 8 freeze-thaw intervals. There was also increased survival in the treatments with less moisture due to the fact that there was less ice formation due to the lack of water that could damage cells.

Weiser and Osterud studied the survival of *E. coli* under various freezing and thawing procedures and attempted to determine the manner in which the death of bacteria during freezing occurs [46]. They demonstrated that extracellular, not intracellular, ice was responsible for the death of bacteria at low temperatures. This is partly due to the observation that protection by substances, such as colloids and sugars, decreases formation of intracellular ice and hence decreases death of bacteria at low temperatures. Also, like Kibbey et al. it was seen that repeated freezing caused more damage than a single freezing or storage in a frozen state [16].

There are many theories as to why *E. coli* levels decrease quickly when cells are added to soil, ranging from nutrient availability to the presence of toxic compounds in the soil. Klein and Casida attempted to determine the controlling factors of *E. coli* survival in natural soil [17]. In one experiment, sterilized soil was added to normal soil with *E. coli* inoculants ranging from 10^1 to 10^7 cells/gram. Except for the samples with lower concentrations of *E. coli*, the addition of sterile soil to the non-sterile soil caused an increase in the survival of *E. coli*. This increased survival is believed to be due to the observation that sterile soil possesses an in-

creased level of available nutrients due to the sterilization process that become available to bacteria when added to soil. Experiments were also performed by adding carbon and nitrogen sources to soils, in the form of glucose and ammonium nitrate. Different combinations of glucose and ammonium nitrate were added to soils with 10^6 cells/gram of *E. coli*, and were incubated at 26°C. The addition of ammonium nitrate, with or without glucose, had no effect on *E. coli* survival, whereas glucose additions had a positive effect on survival. However, in order to allow for the extended survival of *E. coli* in soil, it was necessary to add glucose in excess of levels that are normally found in soils. When glucose was not added to soil, the rate of survival decreased and *E. coli* populations decreased to levels lower than concentrations measured when glucose was added in any amount. Successive additions of glucose to soil allowed for multiplication of indigenous microorganisms but also allowed for maintenance of the *E. coli* population at high levels.

Ostrolenk et al. studied the survival of *E. coli* and fecal streptococci at 7.2°C and room temperature in soils that were unfertilized, artificially contaminated, and fertilized with chicken manure [25]. In the soil fertilized with chicken manure stored at room temperature, fecal streptococci were not recovered after 21 days whereas *E. coli* was still present on the 66th day of sampling. When stored at 7.2°C, both organisms survived 109 days. In the unfertilized soil at room temperature, *E. coli* was not present after the initial sampling but fecal streptococci were still present after 109 days. When stored at the lower temperature, *E. coli* only survived 66 days whereas fecal streptococci survived for 123 days. When the artificially contaminated soil was stored for 160 days, a gradual decrease was seen for both organisms from 1×10^7 per gram to 1×10^2 per gram.

Summary

1. Survival of *Salmonella* and indicators in soil increases under moist conditions.
2. Indigenous microorganisms are important in decreasing survival of *Salmonella* and indicators in soil due to predation and competition.
3. Multiple freeze-thaw conditions cause a decrease in microorganism survival in soil.
4. *E. coli* does not seem to enter a "viable but nonculturable" state in soil.
5. Soil type affects the survival of *Salmonella* and *E. coli*.

SALMONELLA AND INDICATORS IN BIOSOLID AMENDED SOIL

When biosolids are land applied, the soil may provide a final barrier for the prevention of transmission of diseases to humans due to the destruction of pathogens. Lang et al. stated that enteric organisms are poorly adapted for survival in the environment and their survival is influenced by many factors including climate, soil moisture, temperature and sunlight [19]. Experiments were focused on the fate of *E. coli* when soil is amended with biosolids during spring and autumn. The soil used was an acidic sandy loam and the biosolids tested included: a dewatered anaerobically digested sludge, a composted sludge with green waste (CPT), and a thermally dried digested product. Field experiments were conducted on small 1.5m x 1.5m plots, and the biosolids were incorporated immediately into the soil upon addition. Two trials were conducted using bare ground conditions and a few sub-plots in each trial were covered with a perforated polythene film in order to manipulate environmental conditions. Due to the polythene film cover, subsurface soil temperatures were always 4–5°C higher than the temperature of the bare soil, and soil moisture levels were always higher after wetting. The conventionally treated anaerobically digested sludge applied during Trial 1 achieved the US EPA's Class B status whereas in Trial 2 it exceeded this limit. The composted of thermally dried biosolids met the US EPA's Class A standards for biosolids. In enhanced treated biosolids applied to soil, *E. coli* populations declined when moisture was low, and then regrowth occurred after rewetting following rainfall. When soil moisture remained near field capacity and rainfall was not an issue, populations remained fairly consistent. When the soil was covered, temperature and moisture increased, and this extended periods of regrowth due to more favorable environmental conditions. For conventionally treated biosolids, a 2–3 log reduction of organisms and a decrease to background levels was seen within 56 days after application. Lang et al. concluded that pathogens should decline to background levels within 2–3 months, and should be safe for agricultural use after specified waiting periods.

Estrada et al. monitored the survival of enterobacteriaceae, including fecal coliforms and *E. coli*, after the addition of sludge to soil containing 74% sand, 20% alluvial matter, and 6% clay [10]. Three types of sludge were used: (1) anaerobically digested sludge followed by mechanical dewatering; (2) anaero-

bically digested and mechanically dewatered sludge with additional heat-drying; and (3) sludge from a dairy food factory that was aerobically digested followed by gravity thickening. Open air studies and controlled laboratory studies were performed utilizing each type of sludge. For the open air studies, in which temperature and humidity could not be controlled, pots containing soil were mixed with different amounts of sludge (10 t ha⁻¹ and 15 t ha⁻¹). In the open air experiments, soil that had been fertilized with calcium ammonium nitrate one year earlier, was also tested with 15 t ha⁻¹ of sludge. For the laboratory studies, in which temperature and humidity were controlled, only 15 t ha⁻¹ of the sludges were added to the soil.

Under open air conditions, enterobacteriaceae in the heat-dried sludge initially increased within 10 days, and higher concentrations were achieved when more sludge was added to the soil. Within 80 days, regardless of the amount of sludge used, the concentrations decreased to 10³ cfu/gram dry matter. Similar behavior was observed for the enterobacteriaceae in the non-heat-dried sludge, although lower concentrations of enterobacteriaceae were detected after 20 days. Fecal coliform concentrations peaked within 20 days in both sludges, and higher numbers were again observed when more sludge was added to the soil. *E. coli* results were similar to the results for the fecal coliforms, and after 80 days *E. coli* was undetected in most samples. When chemical fertilizer was used in soil, growth of some bacteria seemed to be stimulated, as microorganisms were still detected after 80 days in the samples.

In the laboratory studies, both the enterobacteriaceae and fecal coliforms in the soil containing the dairy plant sludge declined rapidly from 10⁴ CFU/gram dry matter to undetectable levels within 80 days. For these samples, *E. coli* was initially undetected, increased within the first five days, and then decreased rapidly until becoming undetected after day 20. For the mechanically dehydrated sludge, all three organisms dropped in numbers over the first 5 days, with an increase between day 5 and 10, and then a subsequent decrease to undetectable levels after day 40. In the heat-dried sludge, all organisms behaved similarly to the other sludge tested.

From these studies, it was concluded that survival of the *E. coli* was not dependent on climatic conditions, such as temperature and moisture. Even when moisture and temperature conditions were favorable for growth, a decrease in the concentrations of organisms was seen. This led the authors to believe that survival in soil depends more on the physical and chemical conditions of

the soil, and on the microorganisms' ability to survive on soil nutrients. Regardless of the conditions tested, most of the populations of microorganisms were below detectable levels after 80 days, so they concluded that it does not seem as if a problem exists due to fecal contamination of soil.

Gibbs et al. studied survival and potential regrowth of microorganisms in a sandy soil amended with biosolids [13]. Soils were monitored for up to 37 weeks for *Salmonella*, fecal coliforms and fecal streptococci. The plots used for these experiments were fenced from the public, but native animals would still have access to the site. Samples were collected from a depth of 10cm and moisture content and temperatures at the sites were monitored. Original fecal coliforms and fecal streptococci levels were 6.3×10^4 and 2.8×10^4 CFU/gram, respectively. The levels of both indicators dropped below detectable levels within the first 4 to 12 weeks but increased at week 19 following rainfall, which brought the moisture content of the soil from 1% to 22%. Initial *Salmonella* levels were fairly low (0.09 MPN/g) and were undetected between weeks 8 and 29. During week 36 *Salmonella* were again detected at low levels (0.72 MPN/g), which were higher than original concentrations detected. Gibbs et al. state that few previous studies had documented regrowth of *Salmonella*, whereas regrowth of indicator organisms was shown to be a common observation [13]. For example, they referred to research by Hagedorn, in which increases of indicators occurred in soil amended with digested sludge [14]. The regrowth seen here seemed to be due to storm events.

Other research has shown that regrowth of indigenous *Salmonella* does not occur in biosolid amended soil after drying and subsequent rewetting [48]. In laboratory studies, anaerobically digested biosolids were added to a clay loam soil and were incubated at 30C allowing for desiccation to occur. Anaerobically digested biosolids without the addition of soil was used as a control in these experiments. Both indicators and *Salmonella* decreased to low or undetectable levels within a few weeks of incubation in both the control and the amended soil. Indicator concentrations increased following rewetting, whereas *Salmonella* remained undetected in both biosolids and amended soil. The possibility of contamination was not possible in these studies, and so it was determined that regrowth of indigenous *Salmonella* does not occur in biosolids or when biosolids are added to soil. As moisture was not limiting following rewetting, regrowth of *Salmonella* was most

likely suppressed due to competition with coliforms and other microorganisms.

Pepper et al. showed survival and regrowth of indicator microorganisms in laboratory and field studies in a clay and sandy loam soil commonly found in the Southwest [27]. It was shown that total coliforms, fecal coliforms, and fecal streptococci all decreased over time in both clay loam and sandy loam soils in laboratory experiments. Survival decreased with increasing temperature, and survival was always extended in the finer textured soil, which offered greater protection of the organisms. The influence of environmental factors on the survival of indicators was determined in two separate field studies done during fall and spring. In the fall experiment, indicators decreased with decreasing soil moisture, and regrowth was seen following rainfall. In the spring experiments, moisture contents were again correlated with the numbers and regrowth of indicators observed following rainfall. Death of enteric indicator organisms in soil is most likely due to different biotic and abiotic stresses, such as the presence of indigenous soil microorganisms, temperature and moisture changes, and increased clay content.

Many forest soils result in poor-quality forests, and so the use of biosolids is a potential means for improving the quality of these soils. Edmonds determined the length of survival of total and fecal coliforms in systems where sludge was applied to clearcut field areas with gravelly glacial outwash soil [8]. Studies were done in the summer and winter, and samples were taken from the biosolids and the soil beneath the biosolids. For the sludge applied in the summer, the total coliforms decreased initially and then increased during the spring with a subsequent decrease the next summer. Fecal coliform counts fell more rapidly reaching zero in the winter, and then increasing again in the spring, summer, and fall. The total and fecal coliforms in the soil beneath the sludge followed the same pattern but concentrations were generally lower.

When sludge was applied in the winter, concentrations for both indicators dropped to low levels within 62 days. Total coliforms increased during spring and fall and decreased in winter and summer, whereas when fecal coliform concentrations reached zero they only increased once during the spring. In the soil beneath the sludge, fecal coliform levels were essentially zero and total coliform levels were highest in the late fall and winter. Indicator survival was related to biotic and abiotic factors, and regrowth of both organisms seemed to be influenced by moisture increases due to rainfall.

Survival also increases when sludges were initially applied in the summer compared to the winter, probably due to the initial cold temperatures. Indicators were found in the soil beneath the sludge, but the soil was an effective filter and few organisms were found past the first 5 cm.

Summary

1. Studies evaluating the regrowth of *Salmonella* and indicators in biosolid amended soil have shown mixed results.
2. Regrowth and survival of organisms in biosolid amended soil is associated with increased moisture.
3. Survival of microorganisms increases in finer textured soils.

DISCUSSION

Upon reviewing the literature it is apparent that there are contradictory statements that have been made with respect to survival, growth and regrowth of enteric indicator and pathogenic bacteria in environmental samples. Part of the problem may be due to comparisons made between laboratory and field studies; Class B versus Class A biosolid studies; and studies where pathogens in biosolids are monitored following re-inoculation rather than monitoring indigenous populations. In an attempt to defray this confusion we offer relevant definitions for this area of research (Table 1).

Survival and Regrowth in Biosolids and Compost

Previous research suggests growth of *Salmonella* and enteric indicator microorganisms is possible when inoculated into sterile biosolids and compost. This would only apply in situations where contamination occurs from outside sources, such as from birds and other ani-

mals, and when an indigenous population is not present. In this review, we refer to this as recolonization. When *Salmonella* and indicators have been inoculated into non-sterile biosolids and composts, containing an established indigenous population of microorganisms, growth occurred but could not be maintained. Sterilization has been suggested as a means for producing safe biosolids products, but it is likely that a constant indigenous population of coliforms and other organisms is essential for preventing growth of *Salmonella* and *E. coli* introduced from outside sources. More research needs to be conducted in order to determine the actual regrowth potential for indigenous *Salmonella* and *E. coli* and indicators in biosolids and compost after die off and subsequent rewetting. It is still not understood if *Salmonella* actually die, or if they enter a "viable but nonculturable" state, but understanding this phenomenon is crucial in determining if regrowth of indigenous pathogens is possible in land applied biosolids following rewetting.

The survival and potential regrowth of pathogens during storage of biosolids is also an important aspect of biosolids management supported by conflicting views. Some studies showed that storage of biosolids did not seem to be an effective means for decreasing *Salmonella* in biosolids, due to the fact that mature biosolids seemed to support growth of pathogens. Other studies suggest that storage alone can create safe biosolids products if maintained under specific moisture conditions, as moisture and temperature are critical in the survival or inactivation of enteric bacteria in biosolids and compost. Moist environments allow for survival of microorganisms, as moisture is necessary for cells to function properly. By maintaining particular temperatures and moisture, and by monitoring maturity, an indigenous bacterial population could be maintained in the stored biosolids which should help prevent pathogen regrowth or recolonization during storage.

It was often noted that when biosolids and compost samples are initially incubated, an increase in the concentration of organisms can occur. This increase could cause concern if elevated levels are achieved, but the increase is usually followed by a subsequent decrease to levels that would be considered acceptable for land application. Most importantly, more field research is needed for determining survival and the regrowth potential and recolonization for pathogens and indicators in biosolids and compost under various environmental conditions. A summary of the literature regarding survival and regrowth of *Salmonella* and indicators in

Table 1. Relevant terms and definitions for biosolid and land application studies.

Survival	Maintained viability of a microbial population over time.
Growth	Increase in detectable numbers of a known microbial population over time
Regrowth	Increase in numbers after a period of decline in bacterial numbers of indigenous viable bacteria
Recolonization	Reintroduction of bacteria to a substrate (biosolids) followed by growth

Table 2. Summary for *Salmonella* and indicator microorganism survival in biosolids and compost.

Organism	Environmental Factor	Re-inoculated Organisms*			Organisms Indigenous to Biosolids		
		Survival	Growth	Recolonization	Survival	Growth	Regrowth
<i>Salmonella</i>	Moisture Increase**	I	I	I	I	I	NE
<i>E. coli</i>		I	I	I	I	I	I
Indicators		I	I	N	I	I	I
<i>Salmonella</i>	Temperature Increase with Adequate Moisture	N	N	I	D	N	N
<i>E. coli</i>		N	N	I	D	N	N
Indicators		N	N	N	D	N	N
<i>Salmonella</i>	Temperature Increase without Adequate Moisture	D	N	N	D	N	N
<i>E. coli</i>		N	N	N	D	N	N
Indicators		N	N	N	D	N	N
<i>Salmonella</i>	Biotic Competition	D	D	D	N	N	N
<i>E. coli</i>		D	D	D	N	N	N
Indicators		N	N	N	N	N	N

I = increase, D = decrease, N = no data available, NE = no effect, *due to contamination, and **without freezing.

biosolids and compost is presented in Table 2, which clearly shows that there are large data gaps in the literature.

Survival and Regrowth in Soil and Biosolid Amended Soil

Regrowth of enteric bacterial indicators in biosolid amended soil is commonly documented, but experiments done with indigenous *Salmonella* showed that regrowth to only low concentrations was observed. Predation and competition from indigenous microflora is an important aspect affecting the safety of biosolids when applied to land. Enteric bacterial pathogens are not adapted for survival in the soil environment, and so they are often outcompeted or destroyed by predation

from other microorganisms. If a population of indigenous microorganisms in the soil or biosolids is maintained, then growth of enteric bacterial pathogens from contamination should not be an issue of concern.

As with biosolids and compost, many studies have been performed with seeded organisms in soil or biosolid amended soils. Again this only applies to recolonization and more studies need to be done utilizing indigenous pathogens. In soil and biosolid amended soil, survival and regrowth is positively associated with increased moisture. Survival is also associated with finer textured soils and the temperatures during land application. A summary of the literature regarding survival and regrowth of *Salmonella* and indicators in soil and biosolid amended soil is presented in Table 3, which again shows major data gaps.

Table 3. Summary for *Salmonella* and indicator microorganism survival in soil and biosolid amended soil.

Organism	Environmental Factor	Re-inoculated Organisms*			Organisms Indigenous to Biosolids		
		Survival	Growth	Recolonization	Survival	Growth	Regrowth
<i>Salmonella</i>	Moisture Increase**	I	I	N	I	V	V
<i>E. coli</i>		N	N	N	I	I	I
Indicators		I	I	I	I	I	I
<i>Salmonella</i>	Temperature Increase with Adequate Moisture	N	N	N	N	N	N
<i>E. coli</i>		N	N	N	N	I	I
Indicators		N	N	N	I	I	I
<i>Salmonella</i>	Temperature Increase without Adequate Moisture	N	N	N	N	N	N
<i>E. coli</i>		N	N	N	N	N	N
Indicators		D	N	N	D	N	N
<i>Salmonella</i>	Biotic Competition	N	N	N	D	D	D
<i>E. coli</i>		D	D	D	N	N	N
Indicators		D	D	N	D	D	N

I = increase, D = decrease, N = no data available, V = variable effects, *due to contamination, and **without freezing.

A CONCEPTUAL FRAMEWORK FOR REGROWTH

Table 1 illustrates that regrowth can be differentiated from recolonization when different sources of microorganisms are being considered. However, in both cases, an increased number of organisms is observed. Three other parameters appear to determine whether or not overall numbers of a given population increase or decrease. One parameter is clearly the moisture content of the environmental matrix, with microbial numbers often increasing following rainfall events. The second factor seems to be the critical mass of a given indicator or pathogen population surviving at any point in time. Basic soil microbial ecology principles suggest that very small initial microbial populations behave differently in soil than larger populations [21]. Differences in growth characteristics can easily be observed in pure culture, when initial inoculant doses are varied. Specifically, with low inoculant doses, a long lag phase occurs prior to exponential growth. Therefore it is also possible that in environmental samples, a critical pathogen threshold population is a prerequisite for an increase in numbers of a given pathogen population, even if conditions become favorable. If so, the effect of a given environmental parameter may depend on whether the population is above or below the threshold. Finally, the presence of indigenous non pathogenic populations appear to influence pathogen survival and growth. Large populations of indigenous microbes clearly impose biotic stress on pathogens through competition for resources. These concepts are illustrated in Figure 1. Based on Table 3 and Figure 1, rational explanations of cited literature can be made.

Figure 1(a): Under non-stressful soil conditions, the microbial pathogen population survives and growth occurs after a rainfall event.

Figure 1(b) Rainfall event occurs after the pathogen population has decreased beneath the critical threshold required for growth, hence die-off occurs.

Figure 1(c): Rainfall event occurs when the pathogen population has initially decreased but is still at or above critical threshold, hence regrowth occurs.

Figure 1(d): Pathogen population decreases beneath the critical threshold, but reinoculation increases the numbers to above the threshold. Hence following rainfall, recolonization occurs.

Based on these concepts, it may well be that true “regrowth” rarely occurs. However, “recolonization” of

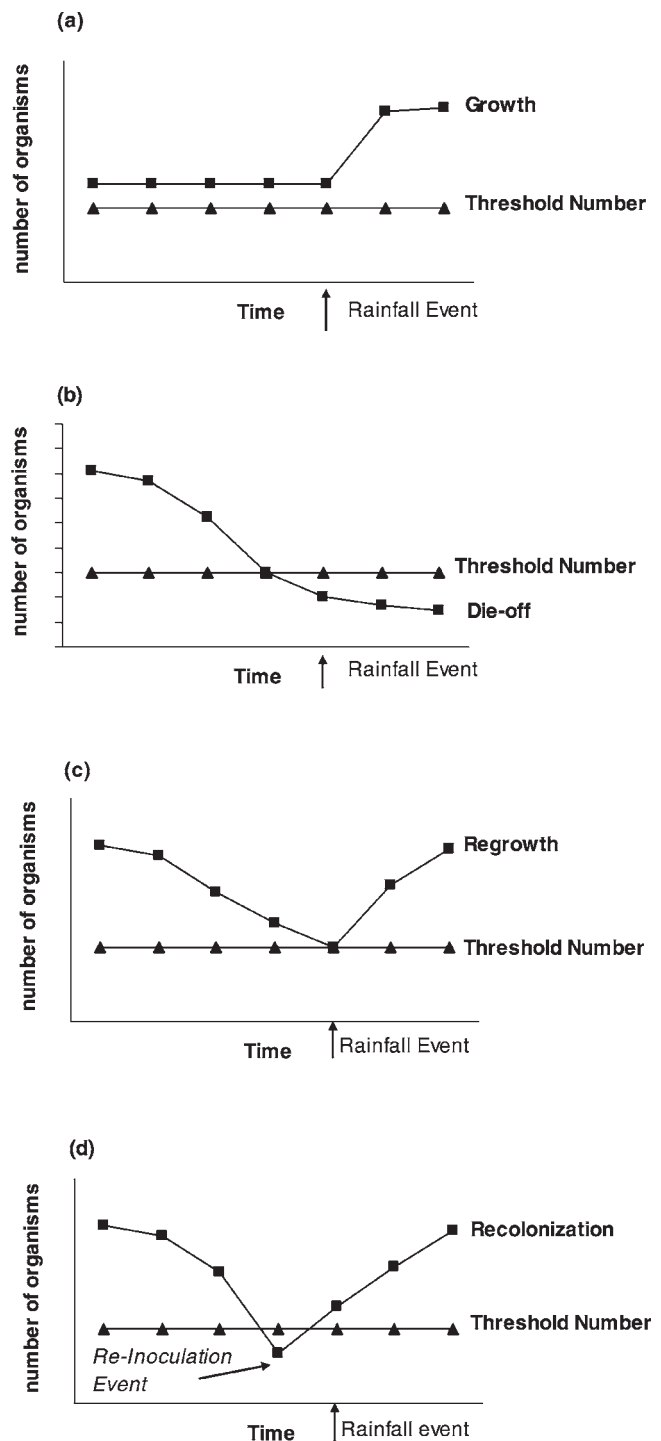


Figure 1. Influence of number of organisms and enhanced moisture on growth, die-off, regrowth and recolonization phenomena.

Class A materials is possible for example during composting of biosolids via windrows. Here “hot spots” of pathogens on the exterior of the windrow can act as a source of reinoculation during the turning and mixing of the windrow. Recolonization can also occur via animal waste contamination. Hence, Class A materials need to be stored carefully, prior to land application. In addition, due to indigenous biotic competition it is very unlikely that regrowth or recolonization of Class B materials occurs, particularly when such materials are land applied.

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REFERENCES

- Ahmed, A.U., and D.L. Sorensen, “Kinetics of Pathogen Destruction During Storage of Dewatered Biosolids”, *Water Environment Research*, Vol. 67, no. 2, 1995, pp. 143–150.
- Bogossian, G., Sammons, L.E., Morris, P.J.L., O’Neil, J.P., Heitkamp, M.A., and D.B. Weber. “Death of the *Escherichia coli* K-12 Strain W3110 in Soil and Water,” *Applied and Environmental Microbiology*, Vol. 62, No. 11, 1997, pp. 4114–4120.
- Brandon, J.R., Burge, W.D., and N.K. Enkiri, “Inactivation by Ionizing Radiation of *Salmonella enteritidis* Serotype *montevideo* Grown in Composted Sewage Sludge,” *Applied and Environmental Microbiology*, Vol. 33, No. 4, 1977, pp. 1011–1012.
- Burge, W.D., Enkiri, N.K., and D. Hussong, “*Salmonella* Regrowth in Compost as Influenced by Substrate (*Salmonella* Regrowth in Compost)”, *Microbial Ecology*, Vol. 14, 1987, pp. 243–253.
- Cardenas, B., “Correlation Between Traditional Stability Parameters of Compost to Turfgrass Phytotoxicity Effects”, MS. Report, University of Massachusetts at Amherst, Department of Civil and Environmental Engineering, 1995.
- Chrostowski P.C., and R.G. O’Dette, “Demystifying the Great Biosolids Debate”, *Pollution Engineering*, Vol. 34, No. 8, 2002, pp. 23–27.
- Davies, C.M., Long, J.A.H., Donald, M., and N.J. Ashbolt, “Survival of Fecal Microorganisms in Marine and Freshwater Sediments,” *Applied and Environmental Microbiology*, Vol. 61, No. 5, 1995, pp. 1888–1896.
- Edmonds, R.L., “Survival of Coliform Bacteria in Sewage Sludge Applied to a Forest Clearcut and Potential Movement into Groundwater,” *Applied and Environmental Microbiology*, Vol. 32, No. 4, 1976, pp. 537–546.
- Epstein, E., and G. B. Wilson. 1975. “Composting raw sludge” In Municipal Sludge Management and Disposal. Information Transfer, Inc., Rockville, Md.
- Estrada, I.B., Aller, A., Aller, F., Gómez, X., and A. Morán, “The Survival of *Escherichia coli*, Faecal Coliforms, and Enterobacteriaceae in General in Soil Treated with Sludge from Wastewater Treatment Plants”, *Bioresource Technology*, Vol. 93, 2004, pp. 191–198.
- Fradkin, L., Gerba, C.P., Goyal, S.M., Scarpino, P., Bruins, R.J.F., and J.F. Stara, “Municipal Wastewater Sludge: The Potential Public Health Impacts of Common Pathogens,” *J. Environmental Health*, Vol. 51, No. 3, 1989, pp. 148–152.
- Gerba, C.P. 2000. “Indicator Microorganisms,” *In Environmental Microbiology*, Maier, R.M., Pepper, I.L., and C.P. Gerba, San Diego, CA: Academic Press.
- Gibbs, R.A., Hu C.J., Ho, G.E., and I. Unkovich, “Regrowth of Faecal Coliforms and *Salmonellae* in Stored Biosolids and Soil Amended with Biosolids,” *Water Science and Technology*, Vol. 35, No. 11–12, 1997, pp. 269–275.
- Hagedorn, C. (1980) Potential Health Hazard Associated With the Disposal of Sewage Sludge on Agricultural Soils in Western Oregon. *Water Resour. Res. Inst.*, Oregon State University, Corvallis, Oregon.
- Hussong, D., Burge, W.D., and N.K. Enkiri, “Occurrence, Growth, and Suppression of *Salmonellae* in Composted Sewage Sludge,” *Applied and Environmental Microbiology*, Vol. 50, No. 4, 1985, pp. 887–893.
- Kibbey, H.J., Hagedorn, C., and E.L. McCoy. “Use of Fecal Streptococci as Indicators of Pollution in Soil,” *Applied and Environmental Microbiology*, Vol. 35, No. 4, 1978, pp. 711–717.
- Klein, D.A., and L.E. Casida, Jr., “*Escherichia coli* Die-out from Normal Soil as Related to Nutrient Availability and the Indigenous Microflora,” *Canadian J. of Microbiology*, Vol. 13, No. 11, 1967, pp. 1461–1470.
- Kuchenrither, R.D., Sharvelle, S., and J. Silverstein, “Risk Exposure: Are treated Wastewater and Biosolids Hazardous to Your Health?”, *Water Environment and Technology*, Vol. 14, No. 5, 2002, pp. 37–40.
- Lang, N.L., Smith, S.R., Bellett-Travers, D.M., Pike, E.B., and C.L. Rowlands, “Decay of *Escherichia coli* in Soil Following the Application of Biosolids to Agricultural Land”, *Water Environment Management Journal*, Vol. 17, No. 1, 2003, pp. 23–28.
- Lewis, D.L., and D.K. Gattie, “Pathogen Risks from Applying Sewage Sludge to Land”, *Environmental Science and Technology*, Vol. 36, 2002, pp. 286A–293A.
- Maier, R.M., Pepper, I.L., and C.P. Gerba. 2000. *Environmental Microbiology*. Academic Press, San Diego, CA.
- Mallman, W.L., and W. Litsky, “Survival of Selected Enteric Organisms in Various Types of Soil,” *American J. of Public Health*, Vol. 41, 1951, pp. 38–44.
- Millner, P.D., Powers, K.E. Enkiri, N.K., and W.D. Burge, “Microbially Mediated Growth Suppression and Death of *Salmonella* in Composted Sewage Sludge,” *Microbial Ecology*, Vol. 14, No. 3, 1987, pp. 225–265.
- O’Dette, R., “Biosolids Found to Pose ‘Negligible’ Risk,” *American City and County*, Vol. 117, No. 4, 2002, pp. 24.
- Ostrolenk, M., Kramer, N., and R.C. Cleverdon, “Comparative Studies of Enterococci and *Escherichia coli* as Indices of Pollution,” *J. of Bacteriology*, Vol. 53, 1946, pp. 197–203.
- Parker, W.F., and B.J. Mee, “Survival of *Salmonella Adelaide* and Fecal Coliforms in Coarse Sands of the Swan Coastal Plain, Western Australia,” *Applied and Environmental Microbiology*, Vol. 43, No. 5, 1982, pp. 981–986.
- Pepper, I.L., Josephson K.L., Bailey, R.L., Burr, M.D., and C.P. Gerba, “Survival of Indicator Organisms in Sonoran Desert Soil Amended with Sewage Sludge,” *J. of Environmental Science and Health*, Vol. A28, No. 6, 1993, pp. 1287–1302.
- Pietronave, S., Fracchia, L., and M.G. Martinotti, “Researchers Analyze How Microorganisms Suppress Pathogen Regrowth,” *BioCycle*, Vol. 43, No. 10, 2002, pp. 57–60.
- Redlinger, T., Grahah, J., Corella-Barud, V., and R. Avitia, “Survival of Fecal Coliforms in Dry-Composting Toilets,” *Applied and Environmental Microbiology*, Vol. 67, No. 9, 2001, pp. 4036–4040.
- Rusin, P., Enriquez, C.E., Johnson, D., and C.P. Gerba, “Environmentally Transmitted Pathogens.” *In Environmental Microbiology*, 2000, Maier, R.M., Pepper, I.L., and C.P. Gerba, San Diego, CA: Academic Press.
- Russ, C.F., and W.A. Yanko, “Factors Affecting *Salmonellae* Repopulation in Composted Sludges,” *Applied and Environmental Microbiology*, Vol. 41, No. 3, 1981, pp. 597–602.
- Schumann, G.L., Soares, H., Holden, C.M., and M.S. Switzenbaum, “Relationship of Traditional Parameters of Compost Stability to

- Turfgrass Quality," *Environmental Technology*, Vol. 14, No. 3, 1993, pp. 257–263.
33. Sidhu, J., Gibbs, R.A., Ho, G.E., and I. Unkovich, "Selection of *Salmonella typhimurium* as an Indicator for Pathogen Regrowth Potential in Composted Biosolids," *Letters in Applied Microbiology*, Vol. 29, No. 5, 1999, pp. 303–307.
 34. Sidhu, J., Gibbs, R.A., Ho, G.E., and I. Unkovich, "The Role of Indigenous Microorganisms in Suppression of *Salmonella* Regrowth in Composted Biosolids," *Water Research*, Vol. 35, No. 4, 2001, pp. 913–920.
 35. Skanavis, C., and W.A. Yanko, "Evaluation of Composted Sewage Sludge Based Soil Amendments for Potential Risks of Salmonellosis," *J. of Environmental Health*, Vol. 56, No. 7, 1994, pp. 19–23.
 36. Soares, H.M. 1995. Pathogen indicator regrowth potential as a method to evaluate compost stability as a method to evaluate compost stability. PhD. Dissertation, University of Massachusetts at Amherst, Department of Civil and Environmental Engineering.
 37. Soares, H.M., Cardenas, B., Weir, D., and M.S. Switzenbaum, "Evaluating Pathogen Regrowth in Biosolids Compost," *BioCycle*, Vol. 36, No. 6, 1995, pp. 70–76.
 38. Sorber, C.A. and Moore, B. E, "Survival and Transport of Pathogens in Sludge-Amended Soil: A Critical Literature Review," 1987, US Environmental Protection Agency, Cincinnati, OH.
 39. Thomason, B.M., Biddle, J.W., and W.B. Cherry, "Detection of *Salmonella* in the Environment," *Applied Microbiology*, Vol. 30, No. 5, 1975, pp. 764–767.
 40. USEPA, 1994, "A Plain English Guide to the EPA Part 503 Biosolids Rule," United States Environmental Protection Agency, Office of Wastewater Management (4204), EPA/832/R-93-003, Washington D.C.
 41. USEPA, 1999, "Biosolids Generation, Use, and Disposal in the United States," United States Environmental Protection Agency, Office of Solid Waste. EPA 530-R-99-099, Washington, D.C.
 42. USEPA, 2000, "A guide to Field Storage of Biosolids and the Organic By-products Used in Agriculture and for Soil Resource Management", United States Environmental Protection Agency, Office of Wastewater Management, EPA/832-B-00-007, Washington D.C.
 43. Van Donsel, D.J., and E.E. Geldreich, "Relationships of *Salmonellae* to Fecal Coliforms in Bottom Sediments," *Water Research*, Vol. 5, No. 11, 1971, pp. 1079–1087.
 44. Van Donsel, D.J., Geldreich, E.E., and N.A. Clarke, "Seasonal Variations in Survival of Indicator Bacteria in Soil and Their Contribution to Storm-Water Pollution," *Applied Microbiology*, Vol. 15, No. 6, 1967, pp. 1362–1370.
 45. Ward, R. L., J. G. Yeager, and C. S. Ashley, "Response of Bacteria in Wastewater Sludge to Moisture Loss by Evaporation and Effect of Moisture Content on Bacterial Inactivation by Ionizing Radiation," *Applied and Environmental Microbiology*, Vol. 41, No. 5, 1981, pp. 1123–1127.
 46. Weiser, R.S., and C.M. Osterud, "The Influence of the Intensity of the Freezing Temperature, Repeated Fluctuations of Temperature, and the Period of Exposure to Freezing Temperatures on the Mortality of *Escherichia coli*," *J. of Bacteriology*, Vol. 50, No. 4, 1945, pp. 413–439.
 47. Yeager, J.G. and R.L. Ward, "Effects of Moisture Content on Long-Term Survival and Regrowth of Bacteria in Wastewater Sludge," *Applied and Environmental Microbiology*, Vol. 41, No. 5, 1981, pp. 1117–1122.
 48. Zaleski, K. J., "Survival and Potential Regrowth of *Salmonella* and Indicator Microorganisms in Biosolids and Biosolid Amended Soil," Masters Thesis. The University of Arizona Department of Soil, Water, and Environmental Science. 2004.