Bioaerosols from the land application of biosolids in the desert southwest USA

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Abstract This study evaluated bioaerosol emissions during land application of Class B biosolids in and around Tucson, Arizona, to aid in developing models of the fate and transport of bioaerosols generated from the land application of biosolids. Samples were collected for 20 min at distances between 2 m and 20 m downwind of point sources, using an SKC BioSampler® impinger. A total of six samples were collected per sampling event, which consisted of a biosolid spray applicator applying liquid biosolids to a cotton field. Each application represented one exposure. Samples were collected in deionised water amended with peptone and antifoam agent. Ambient weather conditions were also monitored every 10 min following initiation of sampling. Concurrently with downwind samples, background (ambient) air samples were collected to compensate for any ambient airborne microorganisms. In addition, biosolids samples were collected for analysis of target indicator and pathogenic organisms. Soil samples were also collected and analysed. Significant numbers of heterotrophic plate count (HPC) bacteria were found in air samples collected during the biosolid application process. These could have arisen from soil particles being aerosolised during the land application process. Aerosolised soil may contribute significantly to the amount of aerosolised microorganisms. Soil particles may be able to more readily aerosolise, due to their low density, small particle size and low mass. Aerosolised HPC bacteria found during biosolids land application were similar to those found during normal tractor operation on non-biosolids applied fields. Coliforms and coliphages were not routinely detected even though they were found to be present in the biosolids at relatively high concentrations, $10^6$ and $10^4$ (dry weight) of biosolids respectively. This could be due to the die-off rate of aerosolised Gram-negative bacteria or sorption to the solid portion of the biosolids. Low numbers of aerosolised coliphages may likewise be due to sorption phenomena. We theorise that only organisms in the aqueous phase of the biosolids were available to desorb and be aerosolised. Animal viruses, which were not detected in the biosolids, were likewise not detected in the aerosol samples. Clostridium perfringens was detected in only a small percent of aerosol samples although it was detected during all weather conditions; other microorganisms were detected during more favourable environmental conditions (relative humidity > 10%). Despite the fact that many of these organisms were present in the biosolids at significant concentrations, their presence in bioaerosols generated during the land application of biosolids was limited to only a small percentage of samples. Bacteria as well as viruses may sorb to biosolids, which contain a high percentage of organic matter, and desorption during land application of biosolids may not readily take place; therefore, these microorganisms may not be readily aerosolised.

Keywords Aerosolisation; bioaerosol; biosolids; pathogen

Introduction

Through the wastewater treatment process, biosolids are a regular byproduct of wastewater treatment. This has caused the industry to continually find new and innovative methods of biosolids disposal. From incineration to land application of biosolids, all methods have met with scrutiny: from the public to upper levels of government (Biosolids, 2002). Land application of biosolids is the latest method of disposal that has come under this scrutiny, although it has been in common use for several decades. Land application of biosolids is a routinely employed method of biosolids disposal in the USA, as 60% of all biosolids produced nationally are disposed of in this way (Biosolids, 2002). Utilising this method, the waste can be recycled for its nutrients, specifically N and P, and used for agricultural crop production. Therefore, land application of biosolids has many benefits but also potential hazards.
Biosolids can potentially contribute heavy metals (such as As, Cu and Zn) to the environment, as well as contributing microbial pathogens (such as viruses, bacteria and parasites). Heavy metals are currently less of an issue than previously, since many studies have been undertaken resolving the issue. In addition, the metal content of biosolids in the US has decreased significantly during the past 10–15 years, due to point source controls (Biosolids, 2002). Microbial pathogens, on the other hand, are still undergoing intense criticism from concerned citizens, although most complaints are of the anecdotal kind. The lack of documented evidence linking biosolids directly to adverse human health effects has not reduced these concerns and it has been stated that “absence of evidence is not evidence of absence”.

Transport of microbial pathogens to groundwater and through bioaerosols is of most concern. This study focused on transport of microbial pathogens via bioaerosols. Viruses, bacteria and fungi can all be easily transferred via bioaerosols. It is known that a bioaerosol is subject to intense physical pressures from the environment (specifically low humidity, ultraviolet and temperature extremes) which tend to inactivate microbes during transport of bioaerosols over long distances. In addition to this, most of these microbes are stressed by conditions present during the biosolids generation process (e.g. mesophilic temperatures, alkaline pH and microbial competition). Previous studies have shown that very few pathogens could be aerosolised through land application of biosolids, but that the potential was present (Sorber et al., 1984). Unfortunately, most of these studies were conducted 20 years ago and have since been followed by only a few investigations. More recently, Dowd et al. (2000) calculated a near 100% bioaerosol viral infection probability, utilising computer-generated models, and predicted values of virus concentrations present in the biosolids, based on data from the 1980s. However, due to the efficiency of modern wastewater treatment plants, these viral biosolid concentrations were much greater than realistic virus concentrations found today.

**Materials and methods**

**Samples**

Bioaerosol samples were collected using six SKC Biosamplers®. This sampler is a modified impinger by which, through centrifugal forces, bioaerosols are collected (impinged) into a 0.1% peptone solution containing antifoam agent B. The samplers were placed in either of two orientations: (a) two sets of three samplers placed perpendicular to the dominant air vector with both sets being placed at two discrete distances, and (b) three sets of two samplers placed perpendicular to the dominant air vector. Both orientations measured aerosols at discrete downwind distances of 2, 3, 10 and 20 m. Each sampler within a set was separated by 1m (Figure 1). These designs allowed the simultaneous collection of multiple samples at as many as three discrete distances under identical environmental conditions. Each sampler was placed at a height of 1.5 m (simulating the average breathing height of a human being) and operated at a collection rate of 12.5 L/min for approximately 20 min. Samplers began operation approximately 2 min prior to exposure to downwind operations, and were operated for a total of 20 min. Background (ambient) air samples were collected off-site where no biosolids had previously been applied. Samples obtained during discing were collected during the operation of a soil-disc harrow (a disc-harrow is commonly used to homogenise soil and remove large clods) on a field where no biosolids had previously been applied. A representative biosolids grab sample was collected in a 1 L Nalgene sample bottle. All samples were placed on ice and transported to the laboratory, where subsequent analysis was conducted within 8 h. Environmental conditions (relative humidity, temperature, wind speed and wind direction) were monitored through the use of a portable weather station. All sample collection bottles, glassware, and collection solutions were sterilised for 15 min at 121°C prior to use.
Operation sites

Samples were collected from sites in and around Tucson, Arizona, where biosolids are routinely applied to cotton fields. Contrary to most metropolitan areas across the US, Tucson land applies anaerobically digested Class B 8% liquid biosolids by the use of a 4,250-gallon Betterbuilt® spray tanker. Most major metropolitan land application operations utilise “cake” (20% biosolids) as it is more economical for large-scale applications. The biosolid spray is released from the tanker at a height of approximately 0.75 m, and reaches a height in the air of approximately 2 m. Large droplets of liquid biosolids are created by the sprayer and fall to the ground approximately in a 3 × 4 m area behind the applicator. Following a 4–6 h drying period, biosolids are subsequently incorporated into the soil using a soil disc-harrow. A sampling event was defined as actual biosolids land application and all air samples subsequently collected 20 min following this event.

Microbial assays

Prior to analysis, all samples were brought to a 23 mL volume with sterile 0.1% peptone solution. Samples were vortexed for 10 s and aliquots for subsequent assays were separated for analysis as follows: 5 mL (total coliform/E. coli), 5 mL (Clostridium perfringens), 4 mL (coliphages), 8 mL (RT–PCR) and 0.3 mL (HPC). A portion of the remaining sample was used for an Aspergillus spp. assay, which was conducted on only a few of the collected samples. Total coliforms and E. coli were analysed using Colilert® and Quantitray®. Clostridium perfringens was plated onto mCP agar after membrane filtration. Coliphage was assayed using the double agar overlay method with TSA agar. Heterotrophic plate count bacteria samples were aliquoted and spread onto R2A medium. Aspergillus spp. assays were performed by spreading onto Czapek and Sabaroud’s Dextrose agars. Human pathogenic viruses were assayed using RT–PCR. Qiagen One-Step RT–PCR kits were used to reverse transcribe and amplify the viral nucleic acid, followed by a second round of PCR to enhance sensitivity. Prior to amplification, viral RNA was extracted using Qiagen viral RNA extraction kits. An 8–10× concentration of the collected air sample was achieved by the use of Centriprep 50® concentrators. RT–PCR was used for the detection of Norwalk-like viruses (NLV) and enteroviruses.

Results and discussion

Air samples collected from a biosolids land application site near Tucson, AZ, demonstrated that HPC bacteria were the group of microorganisms that were aerosolised the most (Figure 2). While HPC bacteria are not normally pathogenic, these organisms could be used initially as relative indicators of the overall presence of aerosolised microorganisms. Much of the aerosolised HPC most likely originated from aerosolised soil, as shown through early field studies. Concentrations of aerosolised HPC during land application of biosolids were similar to the concentrations found during the operation of a standard soil disc-harrow on a non-biosolids applied field (data not shown).
Concentrations were approximately one order of magnitude above that of normal background HPC concentrations, similar to the land application operation. All other indicator microbes were detected in low concentrations downwind of the operation, typically 2–5 orders of magnitude less than that of HPC bacteria. Background concentrations of all indicator bacteria were below detection level, as would be expected. *C. perfringens*, a spore-forming bacterium, was typically found throughout a wide range of environmental conditions, including extreme temperatures, relative humidity, and UV levels (Figure 3). This is as expected, as spore-formers should be the most environmentally stable of the indicator microbes.

Total coliforms and *E. coli* were detected only during conditions of relatively higher humidity (>10%) (Figure 4). Previous studies have shown that Gram-negative bacteria were adversely affected and inactivated, when aerosolised during conditions of high heat, low humidity and elevated UV (Teltsch et al., 1980). These conditions were present during the majority of sampling events, due to the hot arid climate of Tucson, AZ, and could explain the lack of aerosolised indicator and pathogenic microbes.

Coliphage (Figure 5) was only detected in three samples but Norwalk and enteroviruses were not detected in either biosolids or bioaerosols. *Aspergillus* was not detected in any samples, biosolids or bioaerosol (data not shown).

Despite the presence of these indicator microbes in the biosolids (Figure 6), overall detected incidence of the indicators and viral pathogens in the air was limited or below detection limits. Adsorption to solid particles present in biosolids could explain the lack of indicator microbe detection. Current and past studies have shown that viruses specifically adsorb strongly or are embedded within biosolids solid particles and, hence, would not aerosolise easily, whilst those present in the liquid portion of biosolids may aerosolise more easily. This could favour the aerosolisation of microbes during the land application of...
liquid biosolids over that of “cake” biosolids but, due to the large droplet size generated by the liquid sprayer, most aerosolised microbes would probably be contained within these large droplets and not be easily inhaled. It is also worth noting that all samples that were positive for indicator microorganisms were collected within 3 m of the operation. This would indicate the lack of transport of viable indicators.

Conclusions
These data indicated that the land application of biosolids may not pose as great a health risk as originally thought. In this study, the detection of microorganisms generated by the land application of biosolids was minimal or sporadic at best. While HPC bacteria were detected consistently, these microbes were most likely generated by simple operation of the tractor and not from the actual biosolids application. Most microbes aerosolised during land application of liquid biosolids may be trapped throughout the biosolids and not aerosolised readily; even those that did aerosolise were most likely to be contained within large droplets of liquid. These large droplets cannot be inhaled easily. In addition, most pathogens present in biosolids are of faecal origin, and inhalation is not the route of infection most common to these pathogens. Most of these microbes would have to be ingested to cause disease, which is unlikely for microbes contained in relatively large volumes of air. This study is part of a larger nationwide investigation involving comparison of bioaerosols.

Figure 4 Aerosolised total coliforms and E. coli from land-applied biosolids (RH = field relative humidity)

Figure 5 Aerosolised coliphage (host E. coli 15597) concentrations from land-applied biosolids (RH = field relative humidity; TP = field temperature in °C)

Figure 6 Microbial characterisation of biosolids, Ina Road wastewater treatment plant, Tucson AZ (All samples collected from the beginning of February 2001 to the end of June 2001)
generated from spray applications and “cake” biosolids operations under varying environmental conditions. Bioaerosol transport models will be created using data collected from multiple sites across the country, and subsequent risk assessments will be calculated by comparing all operation aspects, including different methods of application and different environmental conditions present on both coasts of the US.

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**References**


