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Field Studies Measuring the Aerosolization of Endotoxin During the Land Application of Class B Biosolids

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Abstract

Endotoxins are a component of Gram-negative bacteria cell walls and are known to be present in biosolids. Endotoxins have been shown to be potent stimulators of the innate immune response causing airway irritation and shortness of breath. Class B biosolids are routinely applied to agricultural lands to enhance soil properties and can be used as an alternative to chemical fertilizers. This study investigated the aerosolized endotoxin dispersed during the land application of Class B biosolids on agricultural land and a concrete surface at two sites in Colorado, USA. Aerosolized endotoxin was captured using HiVol samplers fitted with glass fiber filters, polycarbonate filter cassettes (both open and closed) and BioSampler impinger air samplers. Endotoxins were also measured in the biosolids to allow for correlating bulk biosolids concentrations with aerosol emission rates. Endotoxin concentrations in biosolids, impinger solutions and filter extracts were determined using the kinetic *Limulus* amoebocyte lysate assay. Aerosolized endotoxin concentration was detected from all sites with levels ranging from 0.5 to 642 EU/m³. The four types of sampling apparatus were compared, and the HiVol and open-faced cassette samplers produced higher time-weighted average (TWA) measurements (EU/m³) than the impinger and closed cassette samplers. Ambient wind speed was found to be the variable best describing the observed results with optimal wind speed for highest deposition estimated at 5 m s⁻¹. It is argued that HiVol air samplers are a particularly reliable approach and subsequent analyses relating TWA measurements to wind speed and biosolids characteristics were based on the measurements collected with those samplers.

Keywords

Aerosolized endotoxin; Class B biosolids; Land application; *Limulus* amoebocyte lysate

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

1 Introduction

Biosolids are an end product of sewage wastewater treatment and result from the biological/physical/chemical treatment of sewage sludge. Biosolids can be categorized as either Class A or Class B with respect to pathogens. Class A biosolids are treated to contain pathogens below detectable levels (less than 1000 most probable number (MPN) per gram dry weight (gdw)), while Class B biosolids are treated to reduce fecal coliform counts to $<2 \times 10^6$ MPN per gdw (National Research Council 2002). Each year millions of dry tons of Class B biosolids are applied onto US agricultural land to fertilize and condition the soil. Biosolids are usually stabilized and dewatered before application to land. Stabilization processes include anaerobic digestion, aerobic digestion, composting, lime stabilization or heat drying to reduce odors, vector attraction and pathogens. The most recent national biosolids survey indicated that about 6.5×10^6 dry megagrams (MG) of biosolids were produced in the USA and approximately 60% of the total (i.e., 4×10^6 dry MG) were land-applied to soils in the USA in the year 2004 (NEBRA 2007). Biosolids are known to contain bacteria and viruses, organic and inorganic odor-causing compounds, synthetic organics, heavy metals and biotoxins often greater in concentration than the soils to which they are applied. Regulations restrict public access to application sites based on pathogen content in Class B biosolids. The potential for off-site exposure due to aerosolized biosolids has only recently been considered.

The act of land application of biosolids generates a significant emission of respirable aerosols that can expose downwind communities (Paez-Rubio et al. 2007; Low et al. 2007). It has been reported that residents living near land application sites have experienced acute inflammatory symptoms associated with mucus membrane irritation that subside after a few hours without exposure (Harrison and Oakes 2003). In sensitized individuals, inhalation of bioaerosols has been shown to cause a variety of inflammatory, hypersensitivity and allergic responses in the lungs (Herr et al. 2003). Bioaerosol is the term used to describe airborne microorganisms such as fungi or bacteria, or their by-products, for example endotoxins and glucans (Taha et al. 2006). Endotoxins are lipopolysaccharide molecules from Gram-negative bacteria and cyanobacteria containing a long-chain polysaccharide moiety and a lipid region. Endotoxins are ubiquitous in both indoor and outdoor environments and can be present as whole cells, membrane fragments or macromolecular aggregates of about one million Daltons (Milton 1995). Because endotoxin is a potent stimulator of the innate immune response, the inflammation that it produces can also have deleterious effects (Williams et al. 2005). Response to endotoxins has been demonstrated throughout the respiratory tract from the nasal, paranasal sinuses, pharynx, bronchia and alveoli (Tulic et al. 2004; Viau et al. 2010; Braga et al. 2004). Exposure to airborne endotoxins can cause airway irritation, shortness of breath, chest tightness, cough, decreased lung function and influenza-like symptoms (Rylander 2007). The process that generated the biosolids, such as composting, mesophilic anaerobic digestion and temperature-phased anaerobic digestion, has also been shown to affect the immune response to the respirable particles generated (Viau et al. 2010). Recent interest in the aerosols emitted during land application of biosolids has arisen due to the increased health complaints from residents near land application sites. Public opinion on the application of biosolids to land can vary depending

on perceived health risks, distance of application site from personal property, odor emission and individual knowledge of biosolids issues within the community (Lindsay et al. 2000; Robinson et al. 2012).

Physical and biotic factors can influence bioaerosol characteristics and generation including the physical form of the biosolids, application method and meteorological conditions during application. Some studies have been completed in an attempt to optimize testing conditions such as sampling filter type, filter extraction methods and use of extraction buffers (Douwes et al. 1995; Gordon et al. 1992; Milton et al. 1990; Thorne et al. 1997; Laitinen 1999; Spaan et al. 2007). The literature has shown studies that have addressed the aerosol emissions from biosolids land application sites by measuring levels of bacteria present (Baertsch et al. 2007; Pillai et al. 1996; Brooks et al. 2007). These studies showed detection of bacteria present during biosolids land application which implies the presence of endotoxins. There have been some studies performed that investigated the release of bioaerosols from land application of biosolids and the potential hazards posed by this practice (Pillai et al. 1996; Gerba et al. 2002; Brooks et al. 2004, 2005, 2006). These studies have shown that the release of biosolids bioaerosols has only been documented at the source of aerosol-producing activities such as biosolids spreading, loading or disk incorporation into soils. Bioaerosols can be launched from point, linear or area sources. A biosolids pile is a likely example of a point source, while a field spread with biosolids is an example of an area source (Dowd et al. 2000). There are still significant knowledge and technology gaps including a lack of clear understanding of the fate and transport of bioaerosols, especially within the open environment, an inability to accurately predict the health risks associated with bioaerosolized pathogens, and a lack of standardized bioaerosol sampling protocols, as well as efficacy of samplers (Pillai and Rieke 2002).

The primary objectives of this research were to investigate the endotoxin bioaerosol dispersed during the application of Class B biosolids onto pastureland and a 2-ha concrete drying pad and determine the efficiency of three different air sampling devices: HiVol, filter cassette and impinger (BioSampler). The null hypothesis was that there would be no difference in the estimated endotoxin levels measured using the different air samplers. The research design was constrained by: (1) the accessibility of appropriate biosolids land application sites; (2) meeting state regulatory requirements; and (3) the limited number of commercial scale operators willing to cooperate. These limitations made the field data collected imbalanced by not being able to randomize applications, but representative of commercial field scale operations. Soils are known to contain and release aerosolized endotoxin during normal agricultural activities that disturb the soil. The air quality occurring during the land application process measuring endotoxin levels produced was done utilizing the *Limulus* ameocyte lysate (LAL) endotoxin assay. Studies have shown the LAL assay method to be sufficiently sensitive for measuring low endotoxin levels ($<10 \text{ EU/m}^3$) found in slightly contaminated environments such as outside air (Mueller-Anneling et al. 2004). Since endotoxin has been shown to elicit an immune response when present anywhere in the respiratory system, three different air sampling methods with different air collection volumes and particle size restrictions were examined. The difference in collection efficiency of these three different sampling devices was investigated. Secondary objectives were to determine the effects of lime stabilization on the production of aerosolized endotoxin when

the lime-treated biosolids were land-applied and to determine whether the source of biosolids affected aerosolized endotoxin concentration captured by the sampling devices.

2 Materials and methods

2.1 Field site descriptions and sampler locations

Field experiments were conducted at two types of application sites. The first site, processed in 2010, was on 16 ha of pastureland located in Lamar, CO, (approximately 330 km southeast of Denver, CO) where lime stabilized biosolids were routinely applied to enhance soil condition. The second site, processed in 2012, was a 2-ha concrete pad located near Carr, CO, (140 km north of Denver, CO) where biosolids are routinely applied to “air dry” as part of the stabilization process. Both sites provided generally flat terrain for the biosolids to be applied. The purpose for the two types of surface was to determine whether the deposition of the applied biosolids onto the soil surface of pastureland caused any increase in aerosolized endotoxin over deposition on the concrete pad. Albrecht et al. (2007) recommended a reference or background sample be taken from outside the area of interest assumed to be non-contaminated to determine that endotoxins being detected are generated within a study area. Considering the vastness of the area of the field studies reported here, the upwind aerosolized measured endotoxins were used as background levels.

Ten biosolid samples were evaluated, distinguished by place of origin, how material was processed and the starting endotoxin concentrations (Table 1). All biosolids samples for field application were prepared from activated sludge treatment plants followed by anaerobic digestion. When lime addition was called for, it was done onsite by large-scale weighing and mixing equipment 24 h prior to application to the field sites. The Class B biosolids field application experiments were mixed and applied by a commercial applier from Parker Ag Services (2010) and from the City of Fort Collins, CO (2012). The biosolids were applied to the sites using a Knight side-discharge manure spreader which uses slinging action to distribute the biosolids over an area approximately 15 m in width. The biosolids were surface-applied as a semisolid “cake” form with rate of application at 5 dry MG/ha. The applied test areas were approximately 0.455 ha and in the shape of a rectangle with sides 30 by 150 m (see Fig. 1). Air sampling for each biosolids type was done in triplicate by sampling a “pass” during the application of the biosolids. Each “pass” consisted of the applier starting at the beginning of the row, traveling down the row and returning to the starting position with each “pass” completed in less than 30 min. The applier then returned to the biosolids pile to refill, and the process was repeated until the triplicate “passes” were completed.

The experimental design consisted of four upwind and four downwind sampling stations. The sampling stations were evenly placed from the center of the application field approximately 20 m apart, and they were placed 10 m away from the biosolids downwind edge. Upwind sampling stations were placed at the opposite edge of the application field. This setup allowed for about 40 m of extra biosolids applied to the field on both ends of the monitored field. This was done to alleviate the need to move samplers if there was a slight shift in wind direction. A weather station (Davis Instruments Weather Monitor II, Hayward, CA) was used at each sampling location to measure and record wind speed and direction,

temperature and relative humidity. Wind direction and speed were monitored before, during and after application. Prolonged wind changes of more than 45°, for longer than 2 min or any strong wind gust halted sample collection activities until they subsided. One real-time monitor for particulate matter smaller than 10 µm in aerodynamic diameter (PM10) (DustTrak™ aerosol monitor; TSI, Inc., St. Paul, MN) operated at a flow rate of 1.7 L min⁻¹ and was located at the midpoint of downwind and upwind sample sites to characterize the amount of time the particulate plumes passed the sampler line.

In order to investigate whether low lime addition increases endotoxin levels in the air and biosolids, the following design setup was used. Lime sources and type differed: In 2010 (Lamar, CO), cement kiln dust was utilized, and in 2012 (Carr, CO), quick lime was used. For the Lamar, CO, biosolids samples, triplicate applications of the lime-treated biosolids were performed. For the Carr, CO, sample trials, only the Fort Collins, CO, biosolids sample was used for this experiment. The experimental conditions run were: (1) no lime biosolids; (2) low (1% m/m) lime-treated biosolids; and (3) standard (4.6% m/m) lime-treated biosolids.

2.2 Bioaerosol sampling

Three different air sampling devices were used for this study. Each sampling station consisted of one HiVol sampler (TE-5170 V TSP high-volume air sampler from Tisch Environmental, Cleves, OH), one impinger sampler (BioSampler from SKC Inc. [Item# 225-9595], Eighty Four, PA), and cassette samplers (37-mm three-piece cassette containing a 40-µm polycarbonate filter from Zefron International [Item #PS7345PCEFB], Ocala, FL), one open faced and one closed. For the Lamar, CO, study, only closed cassette air samplers were used. The cassette filters and impingers were clasped on an aluminum tripod and were at approximately 1 m in height. The HiVol sampler is a stand-alone filtering apparatus with air intake at approximately 1 m in height. The impinger uses a liquid sampling fluid with the air being directed at this fluid to trap any particulate matter. The cassette sampler is designed to be used as a closed system with a small opening on each side of the unit for inflow and outflow over the filter. During the 2012 sampling events, one of the cassette samplers had the inflow face removed to allow for the entire surface of the filter to be exposed during sampling to determine whether this had any effect on the trapping efficiency of the filter. The samplers were turned off at the completion of each pass, the filters and impingers changed out and replaced in preparation of the next pass (triplicate passes were done for each biosolids type).

Each HiVol air sampler is equipped with a pump that runs at 1698 L min⁻¹ and was calibrated at the beginning and end of each biosolids type triplicate runs. Calibration was done by following the procedure for calibration contained in the kit TE-5028. The air samples were collected on 20.3 by 25.4 cm glass fiber filters that had been made endotoxin-free by wrapping in foil and baking at 250 °C for at least 1 h (Tsuji and Harrison 1978). After collection of samples, each filter was removed and placed back in the aluminum foil it was baked in and placed in a sealed plastic bag to be frozen at -20 °C. Cassette air sampling was done using a 37-mm three-piece cassette containing a 40-µm polycarbonate filter from Zefron International. The vacuum pumps used (GAST Manufacturing, Benton Harbor, MI)

with a flow rate of 4 L min⁻¹ for each sampling station were calibrated at the beginning and end of each biosolids type triplicate runs. The cassettes are endotoxin-free from the supplier and need no preparation prior to use. Once the air samples were collected, the cassettes were placed in plastic bags and frozen at -20 °C until endotoxin analysis. Impinger air sampling was done using the BioSampler from SKC Inc. (Eighty Four, PA). The pumps (SKC Vac-U-Go) operating at 12.5 L min⁻¹ used for each sampling station were calibrated at the beginning and end of each biosolids type triplicate runs. The BioSamplers were made endotoxin-free by wrapping in aluminum foil and baking at 250 °C for a minimum of 1 h prior to use. Each was filled with 20 mL water containing Tris buffer (0.05 M). The spent impinger liquid was decanted back into a 60-mL borosilicate glass vial and frozen at -20 °C for endotoxin measurement. Research has shown no degradation of samples stored at -20 °C for extended periods of time. Dungan and Leytem (2009) found that even with repeated freeze-thaw cycles, as much as 96% of endotoxin was recovered from field samples. In our study, all samples were processed within 6 months of obtaining them from the field.

2.3 Biosolids pile sampling

The pH of each of the biosolids piles was determined prior to application using a portable pH meter (Orion Star A121) and a flat surface pH electrode (Orion, Model #8235 BN). The flat surface pH probe was firmly pressed against the biosolids surface until a stable reading was obtained. Between readings, the pH electrode was rinsed with distilled water and blotted dry. The lime addition and mixing of biosolids were done approximately 24 h prior to the scheduled application to the plot to allow for equilibration. Three individual samples were removed at equal distances within the large biosolids pile on the site using a clean shovel at a depth of at least 30 cm and then composited. Each composited sample was placed in a one-gallon plastic bag and frozen at -20 °C for endotoxin analysis. From this composite sample, approximately 10 g wet biosolids was dried in an oven at 105 °C for 24 h in triplicate to measure solids percent and determine dry mass. All data were reported on a dry weight basis (Table 1).

2.4 Endotoxin extraction process

All equipment, glassware and liquids used during the endotoxin extraction process were made endotoxin-free prior to use (Tsuji and Harrison 1978). For the cassette filter air samples, the filter holder was opened, the filter removed using forceps and placed in a 60-mL glass vial. For the HiVol sample filters, the foil cover was opened, and a 2.5 by 10.1 cm piece was cut from the filter using a single-edged razor blade and a metal straight edge. To minimize contamination, subsamples from the HiVol filters were made in the laboratory and not in the field. This filter sample was then placed in a 60-mL glass vial for endotoxin extraction. Air filters and solid samples were extracted using 20 mL LAL reagent water (Lonza, Walkersville, MD) plus Tris buffer (0.05 M) (Laitinen 1999) and 0.05% Tween 20 (Spaan et al. 2007). Sample vials were placed on a wrist action shaker for 30 min followed by centrifugation (1000×g) for 5 min to clarify the extraction fluid. For filter samples, 1 mL of the extraction liquid was undiluted or diluted 1:1 using LAL reagent water, while the impinger fluid was used as is for the LAL reaction assay. Biosolids samples required dilutions varying up to 1:100,000 to confirm the measured endotoxin in the sample would be in the standard curve linear range. Lime addition biosolids extracts required a dialysis tubing

treatment (MWCO 3500, Fisher Scientific) with cation exchange resin (Purolite C-100-E, Purolite, Cynwyd, PA) for a minimum of 4 h to remove the excess calcium ions that interfered (color development) with the kinetic LAL assay (Williams 2001).

2.5 Endotoxin concentration determination

The extracts were analyzed for endotoxin concentration using the LAL Kinetic QCL test kit from Lonza (Walkersville, MD). This kinetic test produces a yellow color with time; the greater the color, the higher the concentration of endotoxin present. An aliquot of each extract (100 μ L) after dilution (if necessary) using LAL reagent water was placed in duplicate wells of a pyrogen-free, 96-well microplate (Corning Inc., Corning, NY). Endotoxin standards were prepared from lyophilized *Escherichia coli* O55:B5 using 1:10 serial dilution in LAL reagent water as per the kit instructions. A five-point calibration curve ranging from 0.005 to 50 endotoxin units (EU) mL^{-1} was used with each 96-well microplate assay run ($r^2 = 0.99$). The microplate was incubated for 10 min at 37 °C. Following the incubation, 100 μ L of the LAL Kinetic QCL reagent was added to each of the wells using an 8-channel manual multipipettor. The plate was then placed in the microplate reader (BioTek Instruments, Winooski, VT), and the test was begun. Endotoxin determination was based on the slope of the absorbance (at 405 nm) versus time plot for each microplate well compared with the standard curve. Sample concentrations were reported as EU per mL of eluent, EU per cubic meter of air collected or EU per gdw for biosolids. Quality controls included negative blanks, duplicate samples and positive product controls to determine recovery (–50 to 200%) as described by the manufacturer. Blank filters and BioSampler solutions were monitored for contamination, and the amount of endotoxin from the blank media was subtracted from samples in the same batch. All LAL Kinetic QCL assays were run from the same manufacturer lot number to avoid any bias due to changes in reagents and standards. All replicates gave a coefficient of variation less than 10% using the kinetic LAL test. Ten EUs is approximately equal to 1 ng of endotoxin.

TWA was determined by dividing the measured endotoxin concentration with the time it took to deliver the biosolids to the area for each “pass.” Plume-weighted average (PWA) was determined by dividing the measured endotoxin concentration by the accumulated time under the two peaks measured by the DustTrak™ particulate air sampler for each “pass” (see Fig. 4) (two peaks formed due to the spreader passing the DustTrak™ twice during each “pass”).

2.6 Statistical analysis

Statistical analysis was carried out with the following variables. “Sample” denotes one of the 10 biosolids (7 for Carr, CO, and 3 for Lamar, CO). “Trial” for each sample is numbered 1–3. For each sample and trial, there were measurements at 8 “Locations,” labeled A–H. Of these, 4 (either A–D or E–H) were designated as upwind for a given trial depending on wind direction, and the remaining 4 as downwind, with A corresponding to E, and so on (A was upwind of E or vice versa). The variable “Position” records whether the measurement at a given location was upwind or downwind relative to biosolids application. Additional variables used in regression analysis are identified below.

At each of the 8 locations, TWA endotoxin (EU/m³) was measured with 4 samplers of different types (Carr, CO, study) or 3 samplers of different types (Lamar, CO, study). TWA endotoxin was the response variable in all statistical analyses. Field endotoxin measurements have strong positive skewness, so that analyses may be based on nonparametric tests or outlier-resistant regression methods (Tager et al. 2010). Standard regression and linear modeling methods have been applied to log-transformed field endotoxin measurements in previous studies (Spaan et al. 2008), suggesting a general preference for lognormal over normal distribution assumptions. Use of basic nonparametric tests has been preferred where adequate. Thus, a sign test (nonparametric) was used to compare samplers. For other analyses, the standard methods available are linear modeling methods, which are parametric.

Two primary sets of statistical analyses are reported (in addition to various graphical analyses). The first set provided comparisons of 4 types of measurement devices, separately for upwind and downwind TWA measurements. Coefficients of variation are compared for different samplers, and a sign test (Gilbert 1987) was used to evaluate whether some samplers tended to provide higher TWA values than others. In this application, each of the 4 samplers was compared pairwise to each other sampler (there were 6 statistical comparisons). The N for comparing two samplers was the number of combinations of Sample, Trial and Location where both (upwind and downwind) were measured and where reported response was unequal. The null distribution is that each sampler in a comparison has probability 0.5 of giving the higher measured endotoxin in each such combination. The number of instances where each sampler had the higher value is calculated. The p value for a sign test is computed from the binomial distribution of the number of instances where a particular sample has the higher TWA value. Finally, pairs of samplers were compared based on Spearman's ρ , a rank-based correlation as in Stephenson et al. (2004). If samplers were to be viewed as interchangeable, the pairwise correlations should be positive.

A second set of results dealt with use of regression methods to predict downwind TWA. For the regression analysis, the response variable was logarithm of TWA endotoxin. The independent variables were biosolids sample (categorical) and wind speed, accounting for nonlinear wind speed effects using a spline approach. Details of this methodology follow. Aim of the regression modeling was to account simultaneously for multiple variables that may affect TWA: for example, to evaluate variation among biosolids while accounting for wind speed variations associated with the measurements for different biosolids. TWA endotoxin was transformed to common logarithms for regression analysis. For results based on logarithmically transformed response variables, appropriate transformation of estimated regression coefficients yields estimates of multiplicative effects on geometric mean response (Fox and Weisburg 2011). TWA measurements used for regression were 104 downwind measurements from the HiVol samplers. The HiVol measurements were chosen for regression analysis based on aspects of the previous comparative analysis that suggested favorable performance. Regressors ("Xvariables") used in regression were sample biosolids (categorical with 10 levels) and wind speed (m s⁻¹).

Measurements of pH and biosolids endotoxin concentration were evaluated in separate graphical analyses and not incorporated as X variables in the regression analysis. These variables are potentially affected by biosolids differences such as application of lime.

Various authors (Gelman and Hill 2007) discuss incorporation of X variables into a regression that may be affected by other X variables. If biosolids differences are identified, causal mediation by effects on pH or solid endotoxin may be an explanation for consideration.

A wind speed optimum for biosolids land application has been reported in the previous literature (Baertsch et al. 2007; Dungan and Leytem 2011). The regression approach adopted allows for potential nonlinear wind speed effects by reliance on natural splines (Hastie 1992; Harrell 2001; Ruppert et al. 2003). The approach is akin to the introduction of squares or other powers of an X variable into a multiple regression, i.e., polynomial regression. As with polynomial regression, the approach involves possibly multiple transformations of the original continuous variable, each associated with one model degree of freedom (df). Generally, relatively more complex spline curves (more df) may be considered for relatively more important variables (Harrell 2001). We adopted spline models with 3 df a priori. These can represent simple cases such as linearity but are sufficiently flexible to represent a wind speed optimum, also allowing some asymmetry relative to the optimum; i.e., a curve rising more steeply to one side of an optimum than it falls on the other (or conversely). For a given fitted spline curve, an estimate of the wind speed optimum (the wind speed maximizing TWA) was computed using a grid approach: Predicted TWA was computed from 500 equally spaced values of wind speed (the minimum and maximum observed wind speeds plus 498 intermediate values), and the optimum was the wind speed with highest predicted TWA.

Two options for incorporating biosolids (a categorical variable) into a regression approach are a fixed effects approach and a random effects approach. The fixed effects approach can be considered the more basic approach and is sometimes recommended for preliminary analyses (Zuur et al. 2009). Tests of treatments at the biosolids level such as liming are subject to inflated false positive rates if evaluated in a fixed effects framework, producing the problem of pseudoreplication (Crawley 2007). Statistical and graphical data analysis was performed with R version 3.1 (R Core Team 2013; Crawley 2007). Standard regression diagnostics such as evaluation of residual normality were applied as described by Crawley (2007) and Fox and Weisburg (2011). Mixed linear models were implemented using contributed R package lme4 (Bates et al. 2014; Gelman and Hill 2007; Zuur et al. 2009). Additional basic statistical tests are documented in association with results.

3 Results/discussion

3.1 General

One purpose of this study was an attempt to use biosolids from various sources to determine how these might influence the measured aerosolized endotoxin dispersed during land application. As shown in Table 1, the biosolids used in this land application study were obtained from wastewater treatment plants of varying size (plant flow) and had different solids retention times (14–45 days). All biosolids were formed using activated sludge treatment followed by anaerobic digestion and dewatering. The percent solids present in the biosolids cake were similar and ranged from 19 to 26%, while the percent volatile solids reduction averaged from less than 38 to 64% using the Van Kleeck equation. One striking

difference between the biosolids was the measured endotoxin levels present in the biosolids cake as measured with the LAL endotoxin assay. The NY biosolids (average 9.5×10^3 EU gdw⁻¹) were at least an order of magnitude lower than the levels found in the CO biosolids samples with the FC biosolids found to be the highest (average 5.5×10^5 EU gdw⁻¹). It is not certain why the NY levels were much lower, but this may have been due to the biosolids formation process itself, the long-distance shipping across the country or long-term storage in the enclosed train boxcars. The range of endotoxin present in biosolids samples was also measured by Brooks et al. (2007) who found endotoxin concentrations ranging from 6.1×10^5 to 5.7×10^9 EU g⁻¹ in four Class B biosolids from four US states. Fluctuations in microbial populations can increase or decrease rapidly depending on temperature and humidity conditions as well as the type of material used thus affecting endotoxin levels (Albrecht et al. 2007). The lower levels present in the NY biosolids did not seem to influence the aerosolized endotoxin levels detected using the various air samplers during the field studies (data below). It should be noted that the NY biosolids were only utilized at the Lamar, CO, study site which were applied to agricultural land (vs. the concrete pad at the Carr, CO, study site) where endotoxins present in the soil could have increased the levels detected. Other studies have shown that detected endotoxin levels during land application of biosolids have been due to the dust and particulates formed when biosolids impact the soil surface and not from the biosolids application process itself (Brooks et al. 2005, 2007; Pillai et al. 1996).

The pH values for the various unlimed biosolids ranged from 7.7 to 8.8. While lime treating the biosolids decreased the levels of detected endotoxins present in the cake biosolids especially in the FC biosolids, there were still detectable levels of endotoxins found during air sampling events. The lime addition to stabilize the cake biosolids influenced the measured pH and endotoxin concentration present. As shown in Fig. 2, with the addition of increasing amounts of lime, the pH was shown to increase as would be expected. The normal stabilization treatment pH for lime addition is a pH greater than 12 held for at least 24 h. The FC biosolids approached this level after 24 h (pH 11.6), while the NY biosolids used during the Lamar, CO, field trials with 4.6% lime addition only reached a pH of approximately 10 after 24 h. This could have been due to insufficient mixing or not enough lime added for the total wet mass of biosolids mixed for the field trials. The weighing and lime addition were completed by commercial handlers onsite. The pH values reported were determined directly by taking multiple measurements from the biosolids pile with a flat tip pH probe. From visual observations in the field, the NY biosolids had visible particles of lime present 24 h after mixing. The pH determined at the Lamar, CO, site by the commercial applier using a slurry method demonstrated a pH of 12. In contrast, the endotoxin concentration in the biosolids decreased with increasing lime for the FC biosolids, while the NY biosolids showed an average slight increase with 1% lime addition and falling again at the 4.6% lime addition. The overall change in the endotoxin present in the biosolids was not significantly different from the control for the lime addition treatments.

3.2 Upwind versus downwind sampling location

Generally, downwind TWA measurements were greater than corresponding upwind measurements (for measurements matched on Sample, Trial and Location), as expected if

upwind samples largely reflect background conditions (e.g., endotoxins originating from environmental sources), while endotoxin measured downwind originate largely from the field application of biosolids. This is usually the case in most outdoor endotoxin studies as reported in the literature (upwind vs. downwind) where the upwind samples are used to measure what would be considered “background” levels. As shown in Table 2, the range for the upwind TWA values was relatively high mainly for the HiVol and cassette samplers due to a few outliers. This is obvious when comparing the range of the measured endotoxin levels compared to the quartile range which contains 50% of the data. This was caused when field site traffic occurred upwind mainly at the Lamar, CO, site on a couple of occasions which caused field dust to be generated upwind of the air samplers. The impinger samplers showed the least amount of variation for the upwind TWA measurement. For the downwind TWA endotoxin measured, again the range was high, but as reflected in the quartile range, 50% of the data were in a similar range among the different air samplers. The high coefficient of variance (CV) found reflects the fact that there is much spread within the data for each air sampler. However, the samplers differed significantly in the percent of cases where downwind TWA was greater than upwind TWA: 96/104 (92%) for HiVol, 56/72 (78%) for open cassette, 87/104 (84%) for closed cassette and 68/84 (75%) for impingers. These percent are significantly different ($p = 0.009$, Chi-square with 3 *df*), and the largest contribution to the Chi-square was from the HiVol samplers. The test repeated with the HiVol data excluded was not significant ($p = 0.3$, 2 *df*). A possible interpretation of these differences is that HiVol measurements have lower error uncorrelated with actual endotoxin. This result provides some support for a decision to focus on the HiVol measurements for additional statistical analyses (e.g., of sample and wind speed effects). The higher volume and non-selectivity of the HiVol sampling device argues that an appropriate analysis can be based on data from that device, eliminating sampling device as a variable.

3.3 Air sampling equipment effect and extraction efficiency

Though a number of commercial air samplers are currently available, a standardized efficient bioaerosol sampling strategy is still lacking. This is a particularly significant problem for measuring bioaerosols within outdoor environments. Pillai and Ricke (2002) suggested that the current ASTM-approved method for evaluating bioaerosols within enclosed municipal waste handling facilities is inadequate for measuring bioaerosols in outdoor environments where wind directions and intensity may show significant fluctuations. Published studies have addressed the optimization of collection, storage, transport, extraction and assay methods for measuring aerosolized endotoxins (Spaan et al. 2007, 2008; Thorne et al. 2010). Efficiency of capturing aerosolized endotoxin on filter-type sampling devices is not well known and is an important area for further research (Duquenne et al. 2013), and sampler performance is strongly dependent on particle size and ambient air velocity (Görner et al. 2010). The methods used to measure airborne endotoxins vary greatly from one study to another in the literature. When comparing air sampling devices, it was expected that the impingers might have shown the highest level of endotoxin concentration due to the direct analysis of the impinger fluid. The filters require an extraction step which may have showed lower levels of detection due to extraction efficiency. Stephenson et al. (2004) showed endotoxin levels 2–10 times greater with impingers than with glass fiber or polycarbonate filters, respectively, while comparing the sampling methods at a wastewater

treatment facility. The work of Spaan et al. (2007) showed that the addition of 0.05% Tween 20 to the filter extraction fluid yielded significantly more endotoxin with an overall improvement in extraction efficiency and it was thus included in the extraction protocol used in this study. From their experimental design, Spaan et al. (2007) gave advice for optimal measurement of aerosolized endotoxin by using glass fiber filters, frozen sample storage, extraction of filters including 0.05% Tween 20 with rocking/shaking and freezing of the extracted liquid. Gordon et al. (1992) demonstrated that extraction of endotoxin from filters is not only dependent on the composition of the filter, but also on the type of aerosol being sampled. Environmental factors such as temperature, relative humidity and wind velocity can all affect the aerosolization of endotoxins. The results reported here showed that the HiVol sampler gave the highest result followed by the open cassette, impinger and closed cassette (see Table 2). The cassette filters were polycarbonate in composition which may have contributed to the lower levels of detected endotoxins when compared to the HiVol glass fiber filters due to the efficiency of endotoxin extraction. This was shown using filters contaminated with pure endotoxins in the work of Laitinen et al. (2001). The authors deposited a solution of pure endotoxin on filters made of different materials and let them dry for 90 min at ambient temperature. After extraction, recovery rates of 90, 80, 40 and 5% were found with filters made of glass fiber, cellulose ester, polycarbonate and PVC, respectively.

In side-by-side comparisons of samplers with all measured variables equal, there were some clear differences as indicated by the sign test (see Table 3). HiVol and open cassette samplers tended to produce higher TWA measurements than impinger and closed cassette samplers. All pairwise comparisons were significant at $p < 0.001$ except for two (impinger versus closed cassette, HiVol versus open cassette). The most remarkable difference was between HiVol and closed cassette samplers. HiVol samplers had higher measured TWA in 102/104 comparisons (98%), and the means (arithmetic and geometric) differed fivefold for cases where both were evaluated. All of the Spearman coefficients displayed in the table are statistically significant ($p < 0.05$). Open cassette samplers may be considered the device type least correlated with other types (ρ 0.4–0.6), while the highest correlations involved the HiVol samplers ($\rho = 0.7$) with both closed cassette and impinger samplers.

3.4 TWA model effects

Multiple regression methods were used to account simultaneously for effects of multiple predictor (X) variables on downwind TWA response ($\log_{10}$), for example to evaluate differences among biosolids samples while accounting for differences in wind speed. Predictor variables considered are biosolids (categorical), wind speed (3 df natural spline) and upwind TWA ($\log_{10}$). Given that some effects of research interest may relate to biosolids differences (lime application, site differences), a model that provides valid testing of all effects of interest is a comparatively specialized mixed model, allowing for variation on multiple scales. However, some features of the data of practical importance are evident from a simpler fixed effects model (essentially, a standard multiple regression model). As suggested in the model development protocol of Zuur et al. (2009), our initial regression was such a model. The model provides a characterization of the relative importance of predictors and confirmation of approximate (residual) normality for variation within samples. The

adjusted R^2 was 71% (68% adjusted for model *df*), suggesting that the predictors taken together are informative.

3.5 Wind speed effects

The main factor affecting airborne endotoxin was wind speed; as wind speed increased, there was enhanced suspension and transport of endotoxins. Reliable conclusions for individual variables such as wind speed or biosolids properties generally require accounting for possible effects of other variables. For example, while wind speed measurements ranged from 1 to 7.6 m s⁻¹ over all measurements in these studies, 6 of the 10 biosolid samples were evaluated within a wind speed range not exceeding 1 m s⁻¹. This imbalance was due in part to evaluation of precisely one biosolids type on each day in the Carr, CO, experiment, with wind speeds varying among but not generally within days. In any case, if wind speed is an important predictor, then comparisons of biosolids samples must account for possible evaluation of samples at different wind speeds. Apparently robust conclusions include that wind speed is the most important variable accounting for TWA endotoxin, for variables in the ranges of those in the data evaluated. TWA endotoxin appears to increase with wind speed, up to some wind speed in the range 4–5.5 m s⁻¹ beyond which it is comparatively stable or (at least in the Lamar, CO, subset) actually diminishes with further increases in wind speed (see Fig. 3). While TWA wind curves were not statistically different in the two experiments, the wind speed optimum is evident primarily from the Lamar, CO, data. The shape of the curve at low wind speeds appears comparatively model sensitive, which could affect the use of regression adjustments to compare any samples that were evaluated primarily at low wind speeds. Imbalances in the biosolids samples evaluated at wind speeds below about 2.5 m s⁻¹ contribute uncertainty in the shape of the wind speed curve in that region and thus also uncertainty in biosolids effects for samples evaluated only at low wind speeds. Other studies have shown a positive correlation of airborne endotoxin with wind velocity (Ko et al. 2010; Dungan and Leytem 2011), and Grinshpun et al. (1991) reported that the “aspiration efficiency” of air samplers generally declines under strong wind conditions.

Wind speed appears the most important predictor of increased endotoxin aerosolization levels followed by biosolids category, for variables in the range considered. For wind speed, the range of somewhat greater than 1 for TWA (log10), observed in Fig. 3, suggests that wind speed variation observed in the study is associated with greater than 10-fold variation in geometric mean TWA. The approach generates wind speed-adjusted estimates of geometric mean for each sample. These varied only fivefold, suggesting that biosolids is somewhat less important variable than wind speed. The highest 5 geometric mean TWAs are ordered as MD < No Lime (NY) < Low Lime (NY) < GC < High Lime (NY), suggesting comparatively high downwind TWA values for Lamar, CO, after accounting for wind speed and upwind TWA. Based on a lognormal distribution model and the model residual mean square, 90% of deviations from model predictions are estimated to fall within an eightfold range. The model R^2 was 71%. Standard *F*-tests associated with wind speed (3 *df*) and sample (9 *df*) were significant ($p < 0.001$), while upwind TWA was associated with $p = 0.08$. However, such p -values may be unrealistically small: Accurate statistical significance

statements may require to account for variation on multiple levels (Sample, Trial and Location) as developed below.

A significant technical difficulty in regression analysis is posed by uncontrolled wind speeds, resulting in samples evaluated at different wind speeds (and conversely, some wind speeds possibly represented by a subset of samples), i.e., the data are unbalanced. However, for the characterization of wind speed effects, the optimum of about 5 m s^{-1} was confirmed by applying the same regression model as was applied above, to a comparatively balanced data subset comprising only the 4 biosolids samples that were evaluated over a wind speed range of at least 2 m s^{-1} . Based on this analysis, the estimated wind speed optimum appears to be a robust result. For the initial model, evidence was found of severe collinearity (essentially, too few data relative to the number of predictors and their correlations) based on variance inflation factors (VIFs) (Zuur et al. 2009; Fox and Weisburg 2011). VIFs were large for wind speed (3 *df*, VIF = 17) and biosolids (9 *df*, VIF = 23) suggesting high statistical error in the characterization of wind speed and sample effects. This collinearity appears to be due to the previously indicated wind speed imbalances among samples: VIFs were comparatively acceptable for the balanced subset indicated above (8 for wind speed, 6 for sample). The mixed model approach described below likely mitigates collinearity problems to some degree by fitting fewer parameters (e.g., a variance of biosolids means rather than individual biosolids means). The fitted wind speed curve had a peak (optimum) at a wind speed of 5.1 m s^{-1} (Fig. 3), appearing to rise relatively steeply at wind speeds below the optimum and be relatively more level at higher wind speeds.

3.6 Biosolids liming and application site effects

Two effects were tested in a more complex, mixed model framework, due to measurement at the sample level, namely liming (0, 1.0 or 4.6%) and site differences (NY versus FC). These can be termed “sample-level” variables, meaning that all measurements of a sample-level variable have the same value for a sample. The model allowed random variation on 4 scales: (1) 5 biosolids sources; (2) Samples within biosolids sources (due to 4 samples for Carr, CO, and 3 for Lamar, CO); (3) Trials (1–3) within samples; and (4) Locations (A–H) within sample. (The first level of variation, Locations, is the model residual variance.) The mixed model approach estimates a normal variance for each level. In addition, the model contains wind speed and upwind TWA (log10) included for the fixed effects model. Three additional fixed *X* variables were included for tests of significance, a contrast of 1% lime (2 samples) to 0% lime (7 samples), a contrast of 4.6% lime (2 samples) to 0% lime and a contrast between FC (Carr, CO) samples and NY (Lamar, CO) samples. These differences were tested based on default test generated by R package lme4 and found to not approach statistical significance (the maximum *t* value of 0.95 was for the comparison of 2 sites).

We characterize variation among biosolids sources and samples, based on a modification of the mixed model. The site comparison variable was retained so that variances are interpreted as among samples for a site. The 2 lime variables were removed so that variance estimates includes any variation due to liming. Estimated variances associated with the levels of variation identified are 0.043 (Location, or residual, within trial), 0.044 (Trials within sample), 0 (Samples within biosolids source) and 0.075 (biosolids source within site). Thus,

close to half of total variance is attributed to biosolids source, while any observed sample variation for a single biosolids source is effectively attributed to variation among trials and location. To further characterize variation among samples, we note that for a lognormal distribution with (log-scale) variance 0.075, 75% of the distribution falls within a 2.3-fold range, while 90% distribution falls within an eightfold range. These results again suggest that variation among the samples is substantial, but possibly comparable to variation from wind speed differences (based on our estimates of the latter). Although the model is not parameterized in terms of means, the mixed model generates mean (the so-called best linear unbiased predictions), in the event ordered as $LE < FC < MD < GC < NY$.

To place these results in perspective, the non-significance of the difference between the two sites is not promulgated as evidence that site differences such as substrate (concrete versus soil) are unimportant. As a whole, the statistical results suggests substantial variation among the biosolids evaluations, which may be due to various causes, perhaps some better understood than others. Substrate differences between the two sites might explain the relatively higher TWA estimates for FC biosolids. The non-significance of the comparison may be due to the fact that the site difference is not large relative to differences due to biosolids source, with a single biosolids source evaluated in the Lamar, CO, experiment. For a definitive study of the difference between soil and concrete substrates, the same biosolids sources should be evaluated on both substrates. Such a study would have a higher effective sample size than that of the mixed model implemented here, and the substrate difference would be effectively compared to variation on a smaller scale.

3.7 Air sampling device effects

The study reported here detected different endotoxin levels when different air sampling apparatus were used. HiVol samplers showed the highest levels of endotoxin recovery, whereas impingers and closed cassette samplers the lowest. Our results differed from those of Duchaine et al. (2001) who suggest that differences exist between traditional filter trapping and impingement for measuring bioaerosols and that impingement may result in higher percent recoveries and greater precision. Their study involved measurement of endotoxin levels in sawmills and swine barns where wind effects would have had little influence on the results. Our results showed lower levels of endotoxins detected using the impinger method when compared to the HiVol and open cassette methods. In another study, impingers were found to be as efficient as sampling on glass fiber filters for measuring airborne endotoxins in a wastewater treatment plant (Stephenson et al. 2004). The work of Brooks et al. (2006) studied the endotoxin levels detected during various aspects of land application of Class B biosolids using the impinger method and found that aerosolized endotoxin produced during the slinging operation to be in the range of 5–143 EU/m³. This value is similar to the range found using impingers to detect airborne endotoxin during the present study measured at 0.51–347 EU/m³. Other studies have shown significant evaporation of impinger solution during long-duration sampling (Lin et al. 1999) where sampling time for measuring airborne endotoxins can range from a few minutes to several hours depending on the study. The times for air sampling during this study were always less than 30 min. Sampling duration is closely linked to the overall sensitivity of the method of measurement with the time selected such that a minimum measured airborne endotoxin

concentration is compatible with the expected concentration for the environment under study.

3.8 Endotoxin exposure and particulate plume effects

Information about dose–response relationships between endotoxin exposure and respiratory or other symptoms is difficult to find in the literature. Environmental concentrations of bioaerosols can have high spatial and temporal variability. A paper discussing the pathogen risks associated with applying biosolids to land discussed reports of illness and even death from residents living near land application sites exposed to dusts and water runoff (Lewis and Gattie 2002). Symptoms included burning eyes and lungs, difficulty breathing, and skin rashes followed within days to months by complaints of gastrointestinal, skin and respiratory infections. As reported by Duchaine et al. (2001), environmental air samples represent a snapshot in time and may be poor estimators for the actual concentrations they represent with short-term samples generally more vulnerable to bias due to temporal variation. According to the Dutch Expert Committee on Occupational Standards, an exposure limit of 90 EU/m³ over an 8-h period is recommended (DECOS 2010). Laitinen et al. (2001) reported respiratory complaints were significantly higher when exposure to endotoxin in the air was over 25 ng/m³ (250 EU/m³). Eye symptoms and chest tightness were higher when the concentration of airborne endotoxin was above 150 ng/m³ (1500 EU/m³). The mean levels of TWA endotoxin detected in this study were much lower than these levels (<43 EU/m³), and it is predicted that exposure to nearby residents is miniscule. Onsite workers could reach a threshold level of endotoxin exposure over a work shift and would be at greatest risk. Other studies have shown that levels of airborne endotoxin decrease significantly with increasing distance from the possible source (i.e., land application, disking, biopile formation). Brooks et al. (2006) showed using impingers that aerosolized endotoxin concentrations decreased to near background levels beyond 100 m downwind of biosolids loading and slinging processes. Brooks et al. (2005) also showed that overall residential risks of infection from annual exposures to bioaerosols generated by land application of biosolids proved to be minimal even at distances of 30.5 m downwind of the source. Overall residential risk of infection from exposure to aerosols generated by land-applied Class B biosolids does exist, but appears to be within acceptable limits.

Another aspect of this study was to determine how long an individual sampling station was exposed to the particulate plume generated by land application. A DustTrakTM particulate air sampler monitored the upwind and downwind lines during each application and recorded two brief plumes at downwind line as the spreader passed (see Fig. 4). This indicates that the application of TWA would therefore greatly underestimate the amount of endotoxin an individual could be exposed to since the real exposure value of concern at a land application site would be a short-term exposure limit (STEL). Since endotoxins initiate the innate immune response, this short-term dosing may be of greater importance than TWA. Adjusting our values to a plume-weighted average (PWA) for the impinger sampler, our land application results gave similar levels of endotoxins as Brooks et al. (2006) observed for biosolids loading operations maximum value 1776 EU/m³, average 186 EU/m³ and geomean of 54 EU/m³. Looking at the HiVol sampler PWA, the exposure levels were: maximum value 2045 EU/m³, average of 381 EU/m³ and geomean of 237 EU/m³. Endotoxin exposure is

more of a dose–response problem and then a cumulative exposure problem when looking at land application of biosolids. The levels of endotoxin measured by PWA exceed the values mentioned above by Laitinen et al. (2001) for respiratory complaints.

3.9 Summary

While the volume of studies investigating the land application of biosolids is increasing, many unknowns still exist. These unknowns are primarily a result of the vast differences in geographical conditions, method of land application and types of biosolids that are applied to study sites (Pillai 2007). The fate and distribution of bioaerosols have to be known to accurately predict and quantify the human and animal health risks associated with aerosolized pathogens and endotoxins. The currently available air samplers are designed primarily for bacterial collection and have not been designed for the collection of viruses or endotoxins, and validated data about the efficacy of the different samplers to collect viruses, bacteria and endotoxins are needed. Inhaled endotoxin is an occupational hazard in many settings, but there are no established exposure limits in the USA despite numerous studies conducted to optimize the air capture of work related to endotoxin. Many studies have been completed measuring the endotoxin produced in enclosed work environments. This study is possibly the first study to utilize the HiVol air sampler for measuring airborne endotoxin during the land application of Class B biosolids, and one of a few studies that compare the use of various air sampling apparatuses for measuring endotoxin levels. The work presented here is one attempt at reducing the unknowns by comparing the efficiencies of various air sampling devices, contribution of wind speed to endotoxin recovery, examining the timing of the particulate plume, as well as comparing land application of various biosolids sources at different sites.

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Abbreviations

df	Degrees of freedom
EU	Endotoxin units
Gdw	Gram dry weight
PWA	Plume-weighted average
TWA	Time-weighted average
MPN	Most probable number

LAL	<i>Limulus</i> ameocyte lysate
VIF	Variance inflation factor
STEL	Short-term exposure limit
FC	Fort Collins, CO
NY	New York, NY
MD	Metro Denver, CO
LE	Littleton/Englewood, CO
GC	Greeley, CO

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Figure 1.
Biosolids field application layout.

Figure 2. pH and endotoxin levels as a result of increased lime addition. Dotted lines connect the means for the Lamar, CO samples.

Figure 3.
Predicted endotoxin levels associated with wind speed.

Figure 4.
Example of the particulate matter plume formed during the land application process.

Table 1.

Description of the biosolids used in the land application studies.

WW Treatment Facility [†]	Field Site [‡]	WATER TREATMENT TRAIN			BIOSOLIDS TREATMENT TRAIN			
		Plant Flow (MGD) [§]	Waste Water Treatment [¶]	SRT (Days) [#]	Biosolids Cake (%TS) ^{††}	Biosolids Produced (DMT/Y) ^{‡‡}	Avg %VSR ^{\$\$}	Biosolids Endotoxin conc (EU/gdw) ^{¶¶}
NY	Lamar	NA ^{##}	AS	14	26	25,000	<38%	9.50 E+03
FC	Carr	13.8	AS	45	23	1,900	>43%	5.50 E+05
GC	Carr	8.0	AS	30	19	1,300	54%	9.70 E+04
MD	Carr	133.0	AS	24	21	23,000	59%	9.78 E+04
LE	Carr	21.6	TF/AS	43	19	3,300	64%	1.20 E+05

[†]Wastewater Treatment Facilities: NY = New York City Dept of Environmental Protection;

FC = Fort Collins, CO Drake Water Reclamation Facility; GC = Greeley, CO Water Pollution Control Facility;

MD = Denver, CO Metro Wastewater Reclamation District; LE = Littleton/Englewood, CO Wastewater Treatment Plant.

[‡]Field site where land application occurred - Lamar, CO (2010) or Carr, CO (2012).[§]Million gallons per day[¶]AS activated sludge; TF trickling filter.[#]Solids retention time in days.^{††}Average percent Total Solids in biosolids cake applied to field.^{‡‡}Plant average annual biosolids production in dry metric tonnes per year.^{\$\$}Average percent volatile solids reduction.^{¶¶}Average endotoxin reported as endotoxin units per gram dry weight.^{##}Value not available.

Table 2.

Summary of the endotoxin levels present from the air samplers after application of Class B biosolids.

Variable [†]	Sampler [‡]	n	Mean	Median	Range	Quartile Range	CV [§]
TWA Upwind	hv	104	8.37	3.67	0.10 – 121	1.50 – 6.45	216
	cc	104	4.75	2.08	0.18 – 69.7	1.32 – 3.20	209
	co	72	9.53	3.32	1.14 – 112	2.00 – 5.96	194
	im	85	1.77	1.30	0.00 – 8.18	0.83 – 2.39	83
TWA Downwind	hv	104	42.8	26.40	2.98 – 139	10.2 – 73.6	93
	cc	104	7.95	5.18	0.12 – 42.8	3.20 – 9.02	105
	co	72	39.1	8.50	1.14 – 642	5.52 – 25.2	243
	im	85	28.6	5.81	0.51 – 347	1.38 – 19.4	223

[†]Values given as EU/m³ except for n and CV

[‡]hv = HiVol, cc = closed cassette, co = open cassette, im = impinger

[§]CV = coefficient of variation

Table 3.

Pairwise comparisons of samplers based on downwind TWA endotoxin and instances where both samplers are evaluated.

Comparison [†]	N	Sign Test [‡]			Ratio of Mean TWA [§]		Spearman's ρ
		r	r / N	p	Arithmetic	Geometric	
hv vs. cc	104	102	98 %	<0.001	5.4	4.8	0.66
hv vs. co	72	44	61 %	0.076	0.66	1.4	0.40
hv vs. im	85	71	84 %	<0.001	1.6	4.0	0.66
cc vs. co	71	13	18 %	<0.001	0.18	0.42	0.56
cc vs. im	85	40	47 %	0.66	0.27	0.74	0.49
co vs. im	53	40	75 %	<0.001	2.3	2.8	0.37

[†] Sampler codes are (hv) HiVol, (im) impinger, (co) open cassette, and (cc) closed cassette.

[‡] N (for a sign test) is the number of instances (combinations of Trial, sample, and location) where the two samplers were both evaluated and the measured TWA was unequal. r is the number of combinations, out of the N, where the 1st sampler had higher measured TWA. The p-value is based on the null distribution of r, binomial with probability parameter 0.5. The same N applies to subsequent statistics (means, correlation) except that tied cases are used in the latter.

[§] Ratios are reported of the mean (arithmetic, geometric) for the first sampler over the second, based on the instances where TWA was evaluated for both samplers. The N for the 4th comparison (cc versus co) is 72 rather than 71 because one instance where TWA was tied for that comparison has been included. (N is also 72 for Spearman ρ given in the final column.)