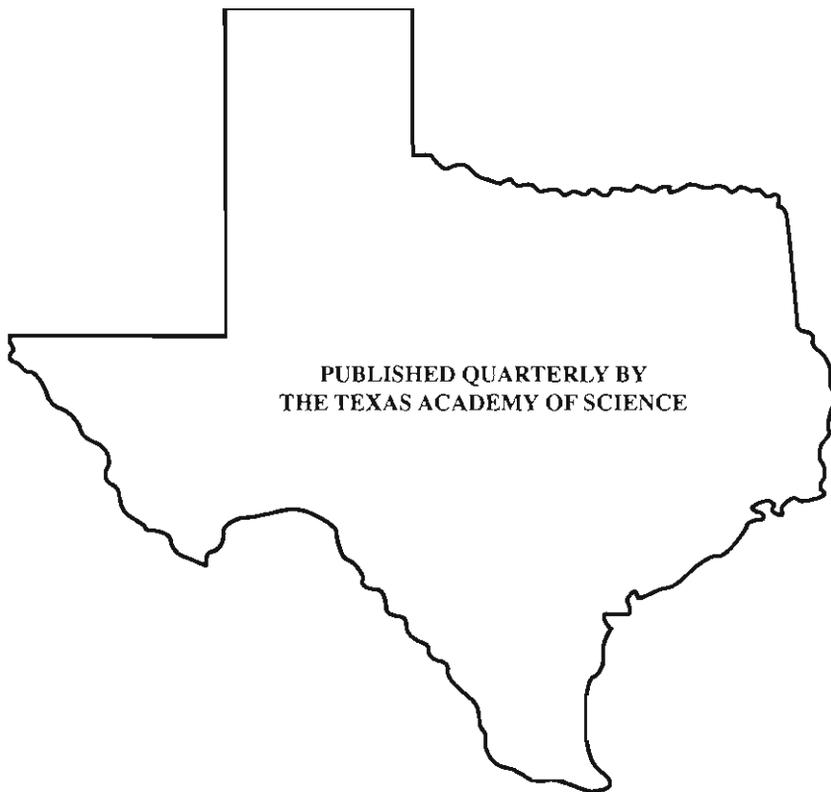


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TWO SMALL TOWNS ON THE SOUTHERN HIGH PLAINS

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Abstract.—Aerosol particulates and bioaerosols were compared between two small cities located in the Southern High Plains. Aerosol particulate generators in rural communities have not been well studied. City A had more than 35 feedyards located in and near it, while City B had one feedyard located beyond the air sampling area. Two sites were located in each of the two cities, and one farm was located downwind of each city. The sites were monitored non-concurrently in the fall. Aerosol particulates were monitored by using PM_{2.5} and PM₁₀ gravimetric monitors; two cyclones air samplers, two laser aerosol monitors, six biological cascade impactors, and a weather station. There were significantly ($P < 0.0001$) higher mean concentrations of PM_{2.5} particulates for City A ($16.48 \pm 1.3 \mu\text{g}/\text{m}^3$ of air) compared to City B ($7.22 \pm 0.7 \mu\text{g}/\text{m}^3$ of air). There were no significant differences in PM₁₀ concentrations between the two cities (City A, $29.97 \pm 2.7 \mu\text{g}/\text{m}^3$ of air and City B, $31.63 \pm 1.7 \mu\text{g}/\text{m}^3$ of air). The cyclone monitor and laser aerosol monitor data indicated higher total concentration of particulates in City B than City A. City A had a significantly ($P < 0.0001$) higher concentration of total microbes $55.7 \pm 3.9 \mu\text{g}/\text{m}^3$ of air compared to City B, $33.9 \pm 2.2 \mu\text{g}/\text{m}^3$ of air. The maximum windspeed was higher and lasted for a longer duration in City B than in City A. It was concluded that the feedyards probably increased the concentration of PM_{2.5} particulates in and around City A. These data may have far reaching implications for cities considering having feedyards located in or around their vicinity.

Particulate aerosols and bioaerosols are currently intense areas of study which concern the public, government regulatory officials and public health officials. Various occupations may contribute to aerosol pollution. Heavy industry was the first to be recognized as contributing to urban air pollution, and this pollution was readily associated with detrimental health effects, especially under certain weather conditions such as occurred in London in 1952 (Logan 1953; Bell & Davis 2001) and Donora, PA in 1948 (Snyder 1994).

Initially, the total suspended particle (TSP) concentration was studied and regulated by the Environmental Protection Agency (EPA) Clean Air Act 1972. However, it soon became apparent that the size of the particles became the focus of the EPA, which set concentration standards in 1987 not to exceed ($50 \mu\text{g}/\text{m}^3$, annually nor $150 \mu\text{g}/\text{m}^3$, 24 h). Then it was recognized that fine particles or respirable particles with a diameter of $< 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) were associated with increased deaths due to heart attacks (Dominici et al. 2000; Samet et al. 2000). In 1996, the EPA published a proposal in the Federal Register to set 24-hour concentration standards for $\text{PM}_{2.5}$ particles at $50 \mu\text{g}/\text{m}^3$ and they received more than 50,000 comments. A final decision was made to set the $\text{PM}_{2.5}$ aerosol concentration standard not to exceed $65 \mu\text{g}/\text{m}^3$ per 24 h on 18 July, 1997. Again, concerns about the $\text{PM}_{2.5}$ standard resulted in proposals to get it lowered (Blodgett et al. 1997). After receiving more than 120,000 written comments, the $\text{PM}_{2.5}$ concentration standard was modified again on 21 September 2006 by reducing the 24-h standard to $35 \mu\text{g}/\text{m}^3$ of air (EPA 2006). Now there is controversy over which is more important for pathogenicity, particle size or the chemistry of the particle (Seagrave et al. 2006). Many areas of the United States have been successful in reducing urban pollution and many are in compliance with the EPA and state regulations governing particulate pollution.

In the last 10 years, agriculture practices (Centner 2001; Horrigan et al. 2002) and concentrated animal feeding operations (CAFO's) have been recognized as major contributors to rural particulate aerosol pollution (Cole et al. 2000; Donham et al. 2002; Centner 2003; Mallin & Cahoon 2003). Rural aerosol pollution falls under the same EPA standards; however, little is known concerning rural particulate generators, concentration of particulates, and the transport of these pollutants (Lee et al. 2006). Even less is known about how to efficiently reduce this particulate pollution, other than the standard practice of wetting it down with water, which can be quite costly (Miller & Woodbury 2003; Miller & Berry 2005).

Most aerosol collection equipment was designed for collection of pollutants in urban settings and not for collecting excessive particulate pollutants from agriculture. Instrumentation for collecting data with tapered element oscillating microbalances (TEOM) may grossly over estimate PM_{10} particulate emissions compared to gravimetric samplers (Vega et al. 2003). There is also an inherent bias of the PM_{10} pre-collectors when sampling aerosols with mass median diameters (MMDs) greater than $10\ \mu m$ in size, which is characteristic of agricultural dust (Busser et al. 2001). The collectors used in this study only sampled city industrial particulate pollution that was $<10\ \mu m$ in size.

The objective of this research was to study particulate air pollution and bioaerosols of two small towns, including one farm downwind of each town in order to determine the effects that a feedyard or feedyards might have on populated areas such as towns or small cities. The hypothesis was that associated feedyards may negatively influence air pollution.

MATERIALS AND METHODS

Sample populations.—Two small towns (City A and City B), approximately 159 km apart with populations of approximately 15,000 were sampled non-concurrently during the fall. Each city was sampled in three locations at approximately the same distance from each site in both cities. Two sites sampled were within the city limits (2.6 km apart) in each city, and one site approximately 11.3 km downwind outside the city limits for each city. The major contrast between the two cities was that City A was near more than 35 feedyards and had an active railroad, while City B had one feedyard downwind, far from any collection sites and had a less active railroad. The distance of aerosol sampling sites from the wastewater plants were as follows: City A sites were 3.8 to 17.8 km from the water treatment sites and City B sites were 2 km to 9.8 km from the wastewater treatment sites. The PM_{10} generated particles do not travel far before they fall to the ground. However, $PM_{2.5}$

particles are capable of traveling long distances before they fall to the ground.

Experimental design.—Property owners granted us permission to set up and maintain monitoring instruments. Air particulate collecting equipment was placed side by side, six meters apart and orientated in a straight line parallel to the prevailing wind direction at each of the three sites in both cities. City A sites were identified as: Chamber of Commerce (C of C), site one, Independent School District (ISD), site two, and Farm, site three; and City B, Lumber Yard, site one, Eagles Lodge, site two, and Farm, site three. These sites were chosen to avoid being too close to buildings and for safety of personnel and equipment during monitoring, and also for their similar distance locations in the two cities.

Air monitoring instruments.—Aerosolized particulates were analyzed by use of high volume ($1 \text{ m}^3/\text{h}$) sequential Andersen Reference Ambient Air Samplers (RAAS-300 series, Andersen Instruments, Smyrna, GA). PM_{10} (Code of Fed. Reg. 1997 appendix K) (two) and $\text{PM}_{2.5}$ (Code of Fed Reg. 1997 appendix L) (two) monitors are stand alone sampling systems that meet the Federal Reference Method (FRM). They provide for multi-filter PM_{10} and $\text{PM}_{2.5}$ sampling. These instruments collect dust on a filter (Whatman Filter Device $2 \mu\text{m}$ PTFE, 46.2 mm, Cole Palmer, Vernon Hills, IL) over a 24-h period (Fed Reg. 1997) and then it rotates to another filter every day sequentially for a total of eight d. The $\text{PM}_{2.5}$ and PM_{10} filters were equilibrated for 24 h in a desiccator's chamber (Oakton Model #35890-00, Cole Palmer, Vernon Hills, IL) with a relative humidity [RH] of approximately 33%. Each filter was identified and its weight recorded after equilibration. This was done prior to the filters' use and again after the collection of ambient particulates. The weighing was done with an analytical balance accurate to $10 \mu\text{g}$ (Denver Instruments, Model #M-220D, Arvada, CO). To load, the filters were placed in a filter assembly (RASS-CASS, Thermo Electron Corp, Environmental Instruments). Then the filter assembly was placed in a metal

transport cassette (RAAS-TC2, Thermo Electron Corp, Environmental Instruments, Franklin, MA) that was taken to the site and placed in the appropriate instrument, and at the appropriate indicated day. The PM_{2.5} WINS impactor (2.5 µm cut-off point) glass filter (Whatman 934-AH 37 mm, Cole Palmer, Vernon Hills, IL) was prepared with one drop of oil supplied by the company and it was replaced every eight days when the RAAS instrument was cleaned. The RAAS air flow rate was maintained at 16.5 L/min and the instrument was recalibrated (RAAS Operators's Manual, Section 8, 8-1-8-45, Andersen Instruments, Smyrna, GA) every three months.

Laser strategic aerosol monitor (SAM) (two).—The laser aerosol monitor (SAM, Model 2005, PPM, Inc., Knoxville, TN), is a real-time, microprocessor based, electro-optical instrument providing mass particulate concentrations (expressed in µg/m³) and optionally, particle sizing distributions that are expressed in nine channels by size (1.25, 3.00, 4.25, 6.00, 8.50, 12, 17, 24, and > 24µm in diameter). The small particle component of sampled air is electro-optically weighed as mass concentration, and the large particle component is sized according to the projected area, and then converted to mass via an algorithm. The SAM flow rate was maintained at 1.5 L/min, and it utilized proprietary automatic calibration and zero methods covered under US Patents. The SAMs collected dust every three min. Total dust was calculated every hour for 24 h, and data were collected for eight days at each of three sites in City A and City B.

Cyclone air sampler.—Two cyclone air samplers (In-Tox Products, Albuquerque, NM) were made of brass piping with slip joints and specifically designed chambers that collected particulates based on their aerodynamic diameters (5.2 µm to 1 µm). The smallest particles (0.32 µm in diameter) were collected onto a filter. Vacuum pumps (Model 1531-320-G557X, Gast Mfg., Benton Harbor, MI) attached to the cyclone devices were calibrated to maintain a flow rate of 28.3 L/min for 24 h. The cyclone intake

orifice height was placed at 1 m. After collection of dust particles, the device was disassembled and the particulates weighed on an analytical balance.

Biological cascade impactors.—Two-stage and six-stage impactors (Andersen Instruments, Atlanta, GA) were used previously to determine the concentration of bacteria, fungi and endotoxin in the air of Southern High Plains feedyards (Purdy et al. 2004). Briefly, the impactors at one-m height were used to collect bacteria for 15 min at 28.3 L of vacuum per minute. An exception, aerobic mesophilic bacteria were collected on brain heart infusion (BHI) medium for 5 min only. This was done to prevent bacterial overgrowth. Culturable microbial concentrations were calculated as colony forming units per cubic meter (CFU /m³ of air) after positive-hole correction (using conversion tables for 200 and 400 hole impactors) for possible multiple microbial particle impactions at the same hole in the multiple-hole sampling orifice plate (Macher 1989).

The cascade impactors were used at each site to determine the size of the viable respirable (< 3 µm in diameter) and non-respirable particles (> 5 to 10 µm in diameter) based on the stage of the device they impacted. Stage-zero of the two-stage impactor and stages 1, 2, 3 and 4 of the six-stage impactor were considered to collect culturable non-respirable bioparticles and stage-zero of the two-stage impactor and stages-five and six of the six-stage impactor were considered to collect the culturable respirable bioparticles. Biological cascade samplers were replicated twice in the AM and twice in the PM for each microbial medium at each City site.

This study cultured specifically for facultative anaerobic mesophilic bacteria, aerobic mesophilic bacteria, and thermophilic bacteria on BHI; enteric bacteria were cultured on brilliant green agar (BGA) and MacConkey agar (MAC). Gram-negative isolates were further identified by API-20E Enteric ID system (bioMerieux, Inc., Hazelwood, MO). *Enterococcus* spp. were cultured on

Enterococcosel agar (ECA). The BHI medium contained a fungal inhibitor (cyclohexamide, 100 mg/L). Fungi were cultured on 5% Malt extract agar (MEA) and Littman oxgall agar (LOA). The MEA medium contained antibiotic inhibitors (streptomycin, 100 mg/L and tetracycline, 5 mg/L). Air flow rates were 28.3 L/min, and collection times were 5 min for BHI, 15 min for BGA, MAC, MEA, LOA, and Enterococcosel agar. Both bacterial and fungal CFU were quantified and reported as CFU/m³ of air. Details on these procedures and methods were previously reported (Purdy et al. 2004). Simultaneously, three two-stage and three six-stage impactors collected in duplicate on each type of medium in the AM and PM at each site. All bacteria were incubated at 37°C for 24 hours, except those on BHI which were incubated at 28°C (mesophilic) for two days. Thermophilic bacteria were incubated at 55°C for 24 hours.

The following parameters for analyzing the biological cascade impactors are reported: (1) various stages of the impactors (two-stage and six-stage), (2) City A and City B, (3) three city sites for each of two cities, and (4) time, AM and PM.

Identification of aerosolized fungi.—Air samples were collected by use of the biological impactors onto Petri plates containing LOA that were cultured to detect airborne mesophilic (28 °C) fungal colonies. Cultures were incubated five days. Fungal colonies were identified (Larone 1995; Watanabe 1994) to the genus level on the basis of gross morphology of a colony, color (top and bottom surfaces), and results of microscopic examination (320X magnification) of hyphae morphology (aseptate or septate), microconidia and macroconidia, and other fruiting structures. Clear 3-cm cellophane tape was bent into a loop with the sticky side on the exterior. The tape was lightly touched to the surface of a fungal colony, and the exposed tape was then placed into a drop of lactophenol cotton blue stain on a microscope slide.

Weather station.—Weather conditions were monitored for each city site by use of a portable weather station (Model Met Data1, Campbell Scientific, Logan, UT) equipped with a 3.5-m tower. The weather station measured wind speed, wind direction, relative humidity, precipitation, soil moisture, air temperature, solar radiation, barometric pressure, and time. The weather station was equipped with a memory storage module. The sampling time occurred at 30-sec intervals and the recording times were 15-min, 1-h, and 24-h intervals.

Statistical analysis.—The experiment was conducted as a completely randomized design with air sample as the experimental unit. $PM_{2.5}$ and PM_{10} gravity data were analyzed with an *ANOVA* by use of mixed linear model analysis for multi-location experimental designs (Littell et al. 1996). City and filter pore size were designated as a fixed effect, and locations were nested within each city. Dust concentrations ($\mu\text{g}/\text{m}^3$ of air) were used to compare $PM_{2.5}$ and PM_{10} levels at locations within each city.

All other data were analyzed by use of a general linear models procedure (SAS 1988). The nested model was used to examine the effect of city, site, and time on the total microbial populations, and on the respirable ($2.5\mu\text{m}$) and non-respirable ($10\mu\text{m}$) diameter size particulate populations. Significant differences between groups were further evaluated by use of the Bonferroni adjusted paired *t*-test. Differences were considered significant at $P \leq 0.05$. Standard error of the mean ($\pm SEM$) was used throughout the study.

The Pearson correlation coefficient was used to analyze the laser strategic aerosol monitors (SAM) hourly particulate ($\mu\text{g}/\text{m}^3$) data and the weather station meteorological parameters (hourly data). The analysis consisted of measuring mean total: particle concentrations, $< PM_{2.5}$ particles and $< PM_{10}$ particles by combining the data of both cities (overall), and individually between City A and City B. The sample correlation coefficient is denoted by *r* and a probability that approaches $P < 0.00$. A similar

analysis was made between RAAS 300 (gravimetric) mean total $PM_{2.5}$ and PM_{10} particulates based on a 24 h gravimetric measurement and correlated with meteorological parameters collected for the same 24 h period.

RESULTS

RAAS data.—The mixed model statement for the $PM_{2.5}$ concentration was significant at the $P < 0.0001$ level and there was a significantly higher $PM_{2.5}$ concentration ($16.48 \pm 1.3 \mu\text{g}/\text{m}^3$) for City A compared to ($7.22 \pm 0.66 \mu\text{g}/\text{m}^3$) for City B. City A and City B $PM_{2.5}$ concentrations were not significantly different among the three collection sites (Figure 1). The only time that the EPA standard for $PM_{2.5}$ ($35 \mu\text{g}/\text{m}^3/24$ hours) was exceeded ($35.46 \mu\text{g}/\text{m}^3$) occurred in City A, site three for one day, and the PM_{10} standard was never exceeded. The mixed model statements for PM_{10} concentrations were not significantly different for City A, $29.97 \pm 1.7 \mu\text{g}/\text{m}^3$, and City B, $31.63 \pm 2.66 \mu\text{g}/\text{m}^3$. There was a significant ($P < 0.004$) difference in PM_{10} concentration among collection sites within each city: City A, site three was $25.87 \pm 2.6 \mu\text{g}/\text{m}^3$ and was less than site one, which was $29.86 \pm 2.6 \mu\text{g}/\text{m}^3$ and site two, which was $36.23 \pm 2.8 \mu\text{g}/\text{m}^3$. City B, site three was $43.61 \pm 5.5 \mu\text{g}/\text{m}^3$ which was greater than site one at $25.24 \pm 3.3 \mu\text{g}/\text{m}^3$, and site two at $24.77 \pm 1.9 \mu\text{g}/\text{m}^3$.

Biological cascade impactors.—There were significant differences in microbial concentrations between the cities for the following bioaerosols: aerobic mesophilic bacteria grown on BHI, Gram-negative bacteria grown on MAC, and mesophilic fungi grown on LOA and MEA. Significant differences were seen among city sites for: anaerobic mesophilic bacteria, aerobic mesophilic bacteria, aerobic thermophilic bacteria (all grown on BHI), Gram-negative bacteria grown on BGA, and fungi grown on LOA and MEA. A significant ($P < 0.0001$) increase ($408 \pm 69 \text{ CFU}/\text{m}^3$ of air) in fungal colonies grown on LOA was observed in the AM compared to the PM (232 ± 42).

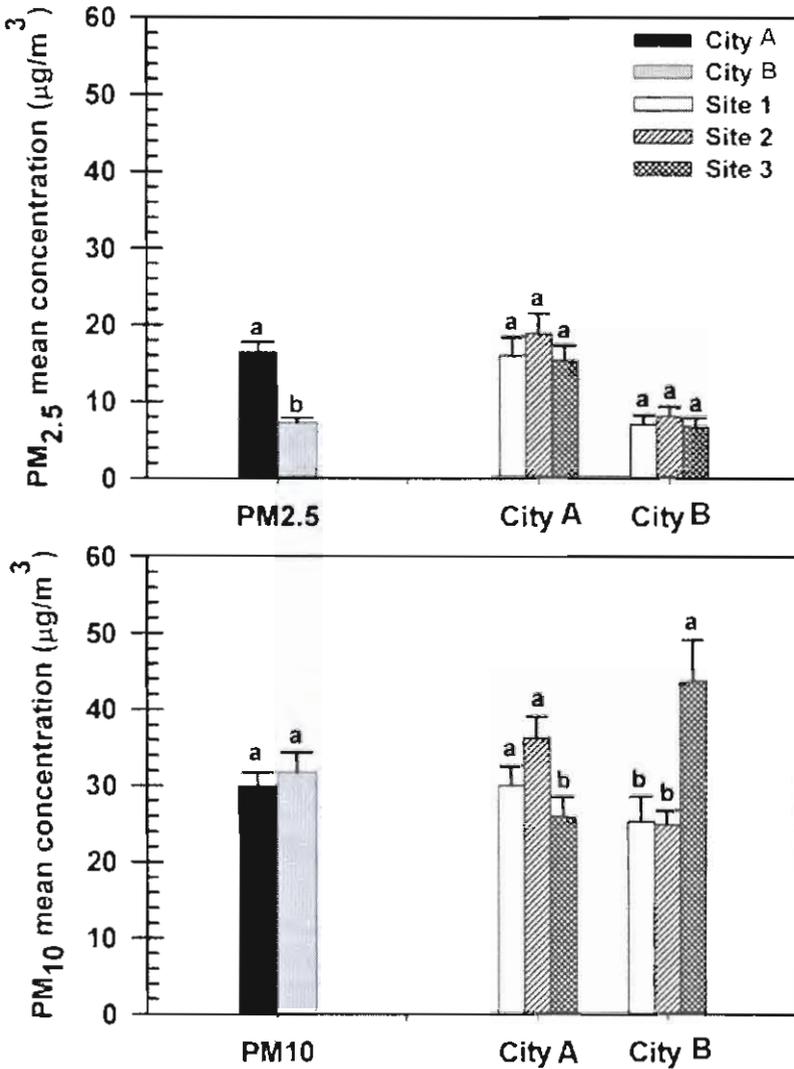


Figure 1. Mean \pm SEM concentration of PM_{2.5} and PM₁₀ particulates are compared between City A and City B and among the three sampling sites in each city. Different under case letters represent significant ($P \leq 0.05$) differences in values.

The mean combined mean total bacterial aerosol (aerobic, facultative anaerobic, thermophilic, Gram-negative, and *Enterococcus* spp.) concentration is shown in Table 1 and the mean

Table 1. Mean bacterial bioaerosols detailed by stage, city, site, and time of day.

Six-Stage Impactor (mm)	Orifice diameter	($P < 0.0001$)	Replications	Mean \pm SEM CFU/m ³
One	1.81	a	24	939 \pm 198
Two	0.91	ab	24	779 \pm 200
Three	0.71	bc	24	480 \pm 72
Four	0.53	bc	24	510 \pm 90
Five	0.34	c	24	378 \pm 74
Six	0.25	c	24	408 \pm 77
<hr/>				
Two-Stage Impactor		$(P < 0.4827)$		
Zero	1.55	a	24	1909 \pm 518
Double zero	0.40	a	24	1419 \pm 463
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Cities		$(P < 0.0630)$		
City A		b	96	683 \pm 120
City B		a	96	1022 \pm 161
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City sites		$(P < 0.0001)$		
City A- site one- Chamber of Commerce		b	32	527 \pm 96
City A- site two- Independent School District		b	32	293 \pm 46
City A- site three- Farm		b	32	1229 \pm 325
City B- site one- Lumber Yard		b	32	1813 \pm 417
City B- site two- Eagles Lodge		a	32	575 \pm 166
City B- site three- Farm		ab	32	680 \pm 90
<hr/>				
Time		$(P < 0.5655)$		
AM		a	96	905 \pm 153
PM		a	96	801 \pm 133

Different small case letters represent significant difference ($P < 0.05$) by Bonferroni pairwise comparisons.

Table 2. Mean fungal bioaerosols grown on malt extract agar detailed by stage, city, site, and time of day.

Six-Stage Impactor (mm)	Orifice diameter	($P < 0.0001$)	Replications	Mean \pm SEM CFU/m ³
One	1.81	b	24	121 \pm 23
Two	0.91	b	24	95 \pm 28
Three	0.71	b	24	130 \pm 20
Four	0.53	a	24	277 \pm 47
Five	0.34	a	24	286 \pm 44
Six	0.25	b	24	54 \pm 15
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Two-Stage Impactor		($P < 0.2501$)		
Zero	1.55	a	24	737 \pm 197
Double zero	0.40	a	24	940 \pm 174
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Cities		($P < 0.0001$)		
City A		b	96	169 \pm 20
City B		a	96	491 \pm 75
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City sites		($P < 0.0001$)		
City A- site one- Chamber of Commerce		c	32	214 \pm 47
City A- site two- Independent School District		a	32	227 \pm 28
City A- site three-Farm		c	32	66 \pm 6
City B- site one-Lumber Yard		b	32	163 \pm 46
City B- site two-Eagles Lodge		c	32	473 \pm 124
City B- site three-Farm		c	32	837 \pm 164
<hr/>				
Time		($P < 0.8219$)		
AM		a	96	335 \pm 55
PM		a	96	325 \pm 59

Different small case letters represent significant differences ($P < 0.05$) by Bonferroni pairwise comparisons.

Table 3. Mean total culturable microbial population compared between biological impactors and City A and City B.

Microbe growth parameter and medium cultured	Mean total \pm SEM CFU/m ³ of air - 2-stage impactor (n=24)	Mean total \pm SEM CFU/m ³ of air - 6-stage impactor (n=24)	Probability 2-stage different than 6- stage impactor	Mean \pm SEM CFU/m ³ of air - City A (n=24)	Mean \pm SEM CFU/m ³ of air - City B (n=24)	Probability that City A different than City B
Mesophilic facultative Anaerobic Bacteria (Brain Heart Infusion Agar)	2502 \pm 729	2403 \pm 377	$P < 0.8973$	1848 \pm 443	3057 \pm 668	$P < 0.1225$
Mesophilic aerobic bacteria (Brain Heart Infusion Agar)	633 \pm 106	912 \pm 218	$P < 0.0978$	756 \pm 213	780 \pm 123	$P < 0.8888$
Thermophilic aerobic bacteria (Brain Heart Infusion Agar)	197 \pm 133	167 \pm 29	$P < 0.8266$	122 \pm 30	242 \pm 132	$P < 0.3730$
<i>Enterococcus</i> (Enterococcosel Agar-aerobic)	1.67 \pm 0.48	1.87 \pm 0.60	$P < 0.7376$	1.28 \pm 0.55	2.26 \pm 0.52	$P < 0.1032$
Gram-negative bacteria (MacConkey Agar)	4.53 \pm 0.89	7.57 \pm 1.63	$P < 0.0857$	4.22 \pm 0.74	7.87 \pm 1.68	$P < 0.0419$
Gram-negative bacteria (Brilliant Green Agar)	3.45 \pm 0.81	7.46 \pm 2.00	$P < 0.0285$	6.19 \pm 2.05	4.71 \pm 0.85	$P < 0.3970$
Fungi (Littman Oxgall Agar)	1584 \pm 310	977 \pm 148	$P < 0.0004$	794 \pm 96	1767 \pm 324	$P < 0.0001$
Fungi (Malt Extract Agar)	1677 \pm 336	962 \pm 147	$P < 0.0008$	675 \pm 80	1964 \pm 321	$P < 0.0001$

total fungal aerosol concentration is shown (Table 2). The mean bacteria and fungi grown under different cultural conditions on different media are compared between the two-stage and six-stage impactors and City A and City B are compared (Table 3).

The efficiency of the two-stage and six-stage impactors was for the most part, not significantly different in collecting the various bacteria. However; the two-stage impactor was significantly ($P <$

Table 4. Mean cultural respirable and nonrespirable microbial populations compared among biological impactors and cities and probability City A differs from City B.

Microbe growth parameter and type of media used	Impactors	Mean total ± SEM Respirable CFU/m ³ of air n=12	Mean total ± SEM Nonrespirable CFU/m ³ of air n=12	Probability (P) that impactors respirable CFU different than nonrespirable CFU	Bioaerosol particle size; Respirable (R) or Nonrespirable (N)	City A mean total ± SEM CFU/m ³ of air n=24	City B mean total ± SEM CFU/m ³ of air n=24	Probability city one CFU different than City B CFU
Mean anaerobic bacteria (Brain Heart Infusion agar)	2-stage	215 ± 30	408 ± 89	< 0.0748	R	160 ± 30	222 ± 29	P < 0.0727
	6-stage	166 ± 29	746 ± 199	< 0.0001	N	596 ± 193	558 ± 112	P < 0.7974
Mean total mesophilic bacteria (Brain Heart Infusion Agar)	2-stage	1038 ± 329	1328 ± 471	< 0.4952	R	451 ± 72	1163 ± 333	P < 0.1335
	6-stage	576 ± 119	1828 ± 309	< 9.0048	N	1397 ± 405	1758 ± 394	P < 0.1891
Mean total thermophilic bacteria (Brain Heart Infusion Agar)	2-stage	164 ± 132	82 ± 26	< 0.3839	R	39 ± 9	166 ± 131	P < 0.4438
	6-stage	41 ± 9	127 ± 24	< 0.3646	N	83 ± 25	125 ± 25	P < 0.1175

Table 4. Cont.

Mean total	2-stage	0.59 ± 0.26	1.1 ± 0.45	<3110	R	0.2 ± 0.1	0.9 ± 0.3	<i>P</i> < 0.2758
<i>Enterococcus</i>	6-stage	0.49 ± 0.2	1.4 ± 0.53	<0.717	N	1.1 0.5	1.4 ± 0.4	<i>P</i> < 0.1558
Spp. (<i>Enterococcosci</i> agar)								
Mean total	2-stage	1.8 ± 0.58	1.7 ± 0.52	< 0.9359	R	2.5 ± 1.0	1.0 ± 0.3	<i>P</i> < 0.5301
gram-neg. (Brilliant Green Agar)	6-stage	1.7 ± 0.44	5.8 ± 1.49	< 0.0033	N	3.7 ± 1.5	3.7 ± 0.8	<i>P</i> < 0.2410
Mean total	2-stage	1.4 ± 0.37	3.2 ± 0.74	< 0.1111	R	1.4 ± 0.4	1.7 ± 0.4	<i>P</i> < 0.5274
gram-neg. (MacConkey agar)	6-stage	1.7 ± 0.44	5.9 ± 1.34	< 0.0003	N	2.8 ± 0.6	6.2 ± 1.4	<i>P</i> < 0.3022
Mean total	2-stage	1113 ± 208	471 ± 131	< 0.0001	R	432 ± 81	1074 ± 219	<i>P</i> < 0.0001
fungi (Littman Oxgall agar)	6-stage	393 ± 95	585 ± 91	< 0.0479	N	363 ± 53	693 ± 143	<i>P</i> < 0.0056
Mean total	2-stage	940 ± 174	737 ± 197	< 0.1330	R	326 ± 58	953 ± 170	<i>P</i> < 0.0001
fungi (Malt Extract agar)	6-stage	340 ± 51	622 ± 109	< 0.0381	N	349 ± 52	1010 ± 197	<i>P</i> < 0.0002

0.0008) more efficient at collecting the fungi cultured on MEA. Concentrations of various bacteria were not significantly different between the two cities, but the fungi were significantly ($P < 0.0001$) 2 to 3-fold higher in City B than in City A (Table 3). The Gram-negative bacterial concentrations were extremely low for both cities.

Respirable and non-respirable mean bioaerosol concentrations of various bacteria and fungi were compared between two-stage and six-stage impactors and between City A and City B (Table 4). The six-stage impactor collected significantly ($P < 0.0001$) more (746 ± 199) nonrespirable facultative anaerobic bacteria compared to respirable (166 ± 29) bacteria, while using the two-stage impactor there was no significant difference between the respirable and nonrespirable bioparticles. Both two-stage and six-stage impactors showed significant differences ($P < 0.05$) between respirable and nonrespirable fungal bioparticles, with the exception of the MEA two-stage impactor. There were no significant differences between bacterial respirable and nonrespirable bioparticles between the two cities. There were significant differences between fungal respirable and nonrespirable bioparticles, and City B had more of both two-stage and six-stage culturable fungal colonies compared to City A.

Fungal identification.—Fungal colonies were identified on all LOA Petri plates that were not too crowded for identification. Identification was completed on 11,432 fungal colonies from both cities; however 210 colonies had no micro- or macro-conidia to help in identification. Twenty-nine genera of fungi were identified from the two cities in descending order, and the top six genera from City A were: *Cladosporium*, 3028, *Alternaria*, 912, *Aspergillus*, 390, *Biospora*, 230, *Rhizopus*, 162, and *Sporothrix*, 37; and from City B were: *Cladosporium*, 3,790, *Alternaria*, 1531, *Chaetomium*, 298, *Penicillium*, 284, *Gliocladium*, 256, and *Aspergillus*, 172.

SAM data.—The nine sizes of particles measured by the SAM instruments were combined into three size categories which are as

follows: $\leq \text{PM}_{2.5}$, $\leq \text{PM}_{10}$, and particles $> \text{PM}_{10}$. The mean total of the three configured particulate size concentrations were $\leq \text{PM}_{2.5}$, 8.7 ± 0.5 , $\leq \text{PM}_{10}$, 11.6 ± 0.5 , and $> \text{PM}_{10}$, $11.6 - 8.7 = 2.9 \mu\text{g}/\text{m}^3$ for City A, and $\leq \text{PM}_{2.5}$, 16.4 ± 3.3 , $\leq \text{PM}_{10}$, 20.2 ± 3.8 , and $> \text{PM}_{10}$, $20.2 - 16.4 = 3.8 \mu\text{g}/\text{m}^3$ for City B.

Cyclone data.—Mean total cyclone particulates were significantly higher ($P < 0.0049$) for City B, $25.1 (\pm 3.0) \mu\text{g}/\text{m}^3/\text{day}$ compared to City A, $18.5 (\pm 2.0) \mu\text{g}/\text{m}^3/\text{day}$. The mean total particulate dust measured by Cyclone 1, $22.5 (\pm 2.6) \mu\text{g}/\text{m}^3/\text{day}$ was comparable to Cyclone 2, $21.1 (\pm 2.6) \mu\text{g}/\text{m}^3/\text{day}$. The mean total dust was significantly different ($P < 0.0011$) among the city sites (Table 5). The ambient air samples from each of the two City farm sites (site three) were considerably dustier than each of the intown sites of the two cities.

Meteorological data.—Meteorological data (air temperature, relative humidity, mean wind speed, wind direction, precipitation, and soil moisture) were collected and summarized at the three sites for each of the two cities (Table 6). The data were correlated with the hourly data measured by the SAM monitors and the hourly meteorological data collected by the weather station. There was great variability as expected, due to the many variables, and significant sample Pearson Correlation Coefficients (r) were very small.

Overall (City A and City B statistics combined) mean total ambient particulates (all different particle sizes combined) SAM concentrations were correlated with wind direction ($r = 0.07$, $P < 0.002$, $n = 2021$), and soil moisture (%v/v) (0.117 , $P < 0.0001$, $n = 1441$). City A, total particulate concentration correlated with wind direction ($r = 0.104$, $P < 0.0013$, $n = 963$), total solar radiation (W/m^2) ($r = 0.333$, $P < 0.0001$, $n = 383$), and soil moisture (%v/v) ($r = 0.166$, $P < 0.0011$, $n = 383$). City B, total particulate concentrations correlated with wind direction ($r = 0.103$, $P <$

Table 5. Cyclone total mean particulate concentration compared between City A and City B /m³ of air detailed by stage, city, and site.

5-Stage cyclone	50% cutoff point (μm)	($P < 0.0050$)	Replications	Mean \pm SEM ($\mu\text{g}/\text{m}^3/\text{day}$)
One	5.2	ab	24	38.3 \pm 6.9
Two	2.1	b	24	17.6 \pm 2.4
Three	1.4	ab	24	24.9 \pm 5.9
Four	0.65	b	24	17.8 \pm 2.9
Five	0.32	b	24	15.5 \pm 2.3
Filter	<0.32	b	24	16.7 \pm 2.4
<hr/>				
Cities		(P < 0.0450)		
City A		b	72	18.5 \pm 2.0
City B		a	72	25.1 \pm 3.0
<hr/>				
City sites		(P < 0.0011)		
City A- site one- Chamber of Commerce		b	24	14.0 \pm 2.6
City A- site two- Independent School District		b	24	13.8 \pm 2.2
City A- site three-Farm		ab	24	27.8 \pm 4.5
City B- site one-Lumber Yard		ab	24	22.7 \pm 4.3
City B- site two-Eagles Lodge		b	24	17.0 \pm 2.8
City B- site three-Farm		a	24	35.5 \pm 7.0
<hr/>				
Cyclones		(P < 0.6778)		
One		a	72	22.5 \pm 2.6
Two		a	72	21.1 \pm 2.6

Different small case letters represent significant difference ($P < 0.05$) by Bonferroni pairwise comparisons.

0.0008, $n = 1058$) and soil moisture (%v/v) ($r = 0.117$, $P < 0.0001$, $n = 1058$).

Table 6. Meteorological data summarized for eight to 12 days at each of three sites for each of two cities. Values are means with ranges in parentheses.

City and Site	Air temp. °C	% Relative Humidity	Wind Speed m/second	Soil Moisture %v/v	Precipitation in mm
City A, Site one- Chamber of Commerce	18.8 (10.5 to 28.2)	63.2 (3.0 to 91.7)	1.2 (0.23 to 3.0)	0.67 (0.53 to 0.729)	0.18 (0 to 0.7)
City A, Site two- Independent School Dist.	20.2 (7.4 to 30.9)	44.9 (9.79 to 89.8)	1.6 (0.2 to 5.3)	0.070 (0.044 to 0.114)	1.8735 (0 to 13.2)
City A, Site three- Farm	22.3 (14.6 to 32.6)	ND	3.1 (0.3 to 6.7)	ND	0 (0 to 0)
City B, Site one- Lumber Yard	7.7 (-3.2 to 25.5)	65.3 (21.1 to 95.4)	2.6 (0.3 to 6.8)	0.94 (0.62 to 0.242)	0.1905 (0 to 2.3)
City B, Site two- Eagles Lodge	12.5 (-1.1 to 30.7)	60.3 (12.1 to 95.4)	2.5 (0.3 to 5.8)	0.465 (0.431 to 0.501)	0.03175 (0 to 0.254)
City B, Site three- Farm	13.4 (-5.0 to 32.2)	41.1 (9.4 to 84.1)	3.8 (0.3 to 11.9)	0.297 (0.245 to 0.5010)	0 (0 to 0)

ND = not done due to damaged probes.

City A, $< PM_{2.5}$ particulate concentration correlated with total solar radiation (W/m^2) ($r = -0.348$, $P < 0.0001$, $n = 383$) and soil moisture (%v/v) ($r = -0.276$, $P < 0.0001$, $n = 383$). City B, $< PM_{2.5}$ particulate concentration correlated with wind direction ($r = -0.109$, $P < 0.0004$, $n = 1058$), and soil moisture (%v/v) ($r = -0.121$, $P < 0.0001$, $n = 1058$). City A, $< PM_{10}$ particulate concentration correlated with air temperature (°C) ($r = -0.146$, $P < 0.0001$, $n = 963$), total solar radiation (W/m^2) ($r = -0.336$, $P < 0.0001$), soil moisture (%v/v) ($r = -0.204$, $P < 0.0001$, $n = 383$), and

precipitation (mm) ($r = 0.189$, $P < 0.0001$, $n = 963$). City B, PM_{10} particulate concentration correlated with wind direction ($r = 0.117$, $P < 0.0001$, $n = 1058$) and soil moisture (%v/v) ($r = 0.204$, $P < 0.0001$, $n = 1058$).

Overall (City A and City B statistics combined) RAAS gravimetric mean: $\text{PM}_{2.5}$ particulates and PM_{10} particulates collected over 24 h periods were correlated with meteorological parameters collected over the same 24 h period. Overall, mean $\text{PM}_{2.5}$ particles were correlated with mean air temperature ($r = 0.404$, $P < 0.0001$, $n = 99$), mean wind speed (m/s) ($r = 0.287$, $P < 0.004$, $n = 99$), and total maximum solar radiation ($r = 0.345$, $P < 0.0021$, $n = 77$). Overall, mean PM_{10} particles were correlated with mean % relative humidity ($r = -0.349$, $P < 0.0019$, $n = 77$), and mean wind speed (m/s) ($r = 0.226$, $P < 0.0232$, $n = 101$). City A mean $\text{PM}_{2.5}$ particles were not significantly correlated with any meteorological parameters and PM_{10} was only correlated with mean air temperature ($^{\circ}\text{C}$) ($r = -0.294$, $P < 0.0277$, $n = 56$). City B mean $\text{PM}_{2.5}$ particles was only correlated with mean % relative humidity ($r = 0.364$, $P < 0.013$, $n = 46$) and PM_{10} was correlated with mean % relative humidity ($r = 0.417$, $P < 0.0044$, $n = 45$), mean wind speed ($r = 0.459$, $P < 0.0015$, $n = 45$), and total precipitation (mm) ($r = -0.295$, $P < 0.0490$, $n = 45$).

DISCUSSION

The toxicity of rural dust compared to urban-generated $\text{PM}_{2.5}$ combustion pollution has not been well studied. Therefore, enforcement legislation for control of rural particulate pollution has been slow, due to the many unknowns about agricultural dust, and to the government's concentration on urban combustion pollution. Thus, there has been a rush to gather data on rural particulate pollution generated by many agricultural practices (Roy & Thorne 2003; Smit et al. 2006; Spaan et al. 2006) and CAFO's in general (Donham 1991; Duchaine et al. 1999; Purdy et al. 2004; Von Essen & Auvermann 2005; Rule et al. 2005).

This study serves as a model to better understand agriculture dust generators and their effect on particle size and transport. For example, it might be of great economic importance for a company deciding to build a multi-million dollar cheese or milk processing plant to know the $PM_{2.5}$ air quality of their desired site. Air handling equipment must be more sophisticated to handle fine particles compared to coarse particles. The integrity of the building must be much tighter to prevent the entrance of fine particles from ambient air. This includes keeping the inner building air under positive pressure and building sophisticated entrance and exit air locks to prevent the entrance of fine particles that may degrade their product. The best choice of location in the present study for an industry that needs better air quality, meaning less $PM_{2.5}$ particles, would be City B.

City B was determined to have the highest total particulate dust concentration during the two-city study, done non-concurrently over 24 d in the fall of the year. However, City A had significantly higher concentrations of $PM_{2.5}$ size particulates which were attributed to the $PM_{2.5}$ dust generated from many feedyards in and around City A compared to City B. This study analyzed the concentration of dust generated by feedyards (Purdy et al. 2007) by subtracting upwind $PM_{2.5}$ background dust from downwind $PM_{2.5}$ feedyard dust over a total of 8 d in the summer and 8 d in the winter in three feedyards. These data show that three feedyards contributed $PM_{2.5}$ dust in the mean amounts of 4.20, 12.18, and 18.18 $\mu\text{g}/\text{m}^3$ of air averaged over 16 d. This size dust does not settle out easily and may remain in the air for long periods of time. Seedorf (2004) reported that areas in Germany with the highest concentration of respirable particles were from three animal-dense areas, Grafschaft Bentheim, Cloppenburg, and Vechta.

Another possible contributor of $PM_{2.5}$ particles for City A, in contrast to City B would be diesel combustion from trains. This type of $PM_{2.5}$ particles would be expected to leave the gravimetric RAAS filters black. A black filter was collected on one occasion at

City A, site two which was attributed to diesel combustion particles from the trains. Several black filters from City B, site three were also collected. It was later determined that a carbon black plant was located some distance from the Farm site three of City B. It should be noted that there was no significant difference in $PM_{2.5}$ concentration between sites in either city; however, the concentration was uniformly high among sites in City A and uniformly low among sites in City B.

Feedyards also contribute PM_{10} particulates to the air (Purdy et al. 2007). For example, it was determined that immediately downwind from four feedyards the following mean PM_{10} concentrations were generated (272.24, 274.84, 139.00, and 29.63 $\mu\text{g}/\text{m}^3$ of air). However, these particles will settle out of the air very quickly and may not have any effect one km downwind. It was assumed that feedyard PM_{10} dust concentration had no effect on this two-city study. It is interesting to note that there was no significant difference in the concentrations of PM_{10} particulates between the two cities, although there were significant differences between the sites in both cities. The Farm site of City B had the highest PM_{10} concentration ($43.61 \pm 5.5 \mu\text{g}/\text{m}^3$ of air) and this farm had more pasture and prairie than the City A farm (PM_{10} concentration $25.87 \pm 2.6 \mu\text{g}/\text{m}^3$ of air), which was surrounded by cultivated land. The prairie contributed much of the PM_{10} concentration and this difference can be seen (Table 2) in fungal CFUs ($837 \pm 164 \text{ CFU}/\text{m}^3$ of air) of farm site 3 of City B, compared to farm site 3 of City A ($66 \pm 6 \text{ CFU}/\text{m}^3$ of air). There is little difference in mean total bacteria between the two sites (Table 1). As long as residents have a healthy immune system and are not allergic to specific fungi, the fungal CFUs reported here are not harmful to humans.

It appears that the bioaerosol data generated in the two cities probably originated more from the PM_{10} particles and not the $PM_{2.5}$ particles. The bioaerosols collected were more concentrated in City B, therefore; bioaerosols do not appear to be associated with the

more concentrated $PM_{2.5}$ dust identified in City A. This is in agreement with Seino et al. (2005), as they reported bacterial aerosols were significantly associated with particles in the PM_{5} range but not finer particles. A second report (Boreson et al. 2004) indicated that biological loading increases with an increase in coarse PM concentration. The higher wind speed (11.9 m/second) encountered at the City B farm may have influenced the higher fungal CFUs compared to those cultured at the City A farm where the wind speed was 6.7 m/second (Table 6).

For City B, the SAM recorded approximately twice the $PM_{2.5}$ and PM_{10} size particle concentrations compared to City A and the larger particle (PM_{10}) concentrations were very similar for both cities. The SAM appears to have underestimated the $PM_{2.5}$ particle concentration of $8.7 \mu\text{g}/\text{m}^3$ of air for City A, compared to the RAAS $PM_{2.5}$ gold standard of $16.48 \mu\text{g}/\text{m}^3$ of air. The particulate concentrations, as determined by the Cyclone air monitors, were higher (City A, $18.5 \mu\text{g}/\text{m}^3$ of air, and City B, $25.1 \mu\text{g}/\text{m}^3$ of air) than that generated by the SAM ($<PM_{2.5}$ for City A, $8.7 \mu\text{g}/\text{m}^3$ of air and City B, $16.4 \mu\text{g}/\text{m}^3$ of air).

The SAM data (total range of particulates, $< PM_{2.5}$ and $< PM_{10}$) were used to determine the sample Pearson Correlation coefficient (r). The SAM instrument had the capacity for collecting hourly concentrations whereas the RAAS 300 gravimetric instruments gave 24 h measurements which were paired with the hourly meteorological weather station data. The numbers of observations were fewer for City A ($n = 963$ at site 1, and 383 at site 2) compared to City B ($n = 1058$) because of missing weather observations due to instrument failure. The soil moisture parameter appeared to give the most significant consistent correlation with particulate dust based on the SAM data. City A had a negative correlation for the following dust parameters: total particulate concentration, $\leq PM_{2.5}$ concentration, and for $\leq PM_{10}$ concentration; while City B was positive by correlation for the same three parameters. It is assumed this was in part because City A sites

received more rain and irrigation water compared to City B sites. However, there are many other factors such as soil type, vegetation cover, wind direction, and wind speed that can influence these correlations. The RAAS 300 gravimetric data for $PM_{2.5}$ and PM_{10} were also used in determining the sample Pearson Correlation coefficient (r) for 24-h periods. The r values, when significant, were larger than those generated by the laser particulate data correlated with the meteorological data. The only significant commonality between the overall $PM_{2.5}$ and PM_{10} data that correlated with the meteorological parameters was wind speed (m/s). Weather conditions, other than wind speed, are important when the formation of particulates are examined, but there was no significant difference ($P > 0.487$) in percent humidity between City A (64%) and City B (57%) (Table 6). There were significant differences ($P < 0.0001$) in temperature (City A, 20.8 degrees C and City B, 10.3 degrees C) and ($P < 0.001$) wind speed between City A (2.13m/s) and City B (2.95 m/s). The meteorological data were similar in some cases during the time of collection but not identical. There were also instances where the meteorological data were different during the time of collection. It is not believed that it was the weather on those particular days that affected the particulate counts. There were no significant differences in the % humidity between City A and City B (Table 6), and it is humidity that is important in particle formation.

Total mean bioaerosols (bacteria and fungi) were significantly ($P < 0.0009$) more concentrated in City B (1513 CFU/ m^3 of air) compared to City A (852 CFU/ m^3 of air) during the study. This held true for the total mean bacterial concentration for City B (1022 CFU/ m^3 of air) compared to City A (683 CFU/ m^3 of air), and for total mean fungal concentration in City B (491 CFU/ m^3 of air) compared to City A (169 CFU/ m^3 of air). The cyclone concentration data are similar to the RAAS $PM_{2.5}$ data for City A. However, cyclone monitor derived data for City B are 3-fold higher than the RAAS data. This difference may be due to the increased wind during sampling at City B.

In conclusion, the many associated cattle feedyards of City A appeared to have significantly increased $PM_{2.5}$ fine dust concentration. This fine dust would not be expected to settle out of the air. There were no significant differences between the two cities in concentration of PM_{10} particulates, but there were significant differences between sites. These site differences can be explained by the fact that PM_{10} particles settle out rapidly. The fungal CFUs were much higher on City B Farm than City Farm one. This could be due to the presence of prairie grasses compared to City A Farm which consisted mainly of cultivated soil; however there is nothing in the literature regarding this phenomenon. The prairie grasses, especially when wet, would supply a food source that would allow for the multiplication of fungal spores. The two most numerous genera of fungi isolated from both cities were *Cladosporium* and *Alternaria*. Both *Cladosporium* (with an average diameter of 3 μm) and *Alternaria* (average diameter of 5 μm) are respirable (Larone 1995). The most numerous aerosolized bacteria CFUs in both cities were aerobic mesophilic bacteria. There were more non-respirable bacterial and fungal CFUs in both cities compared to respirable CFUs. Microbes of the same genera were identified on all stages of the biological cascade impactors. This indicates that they were traveling on particles of varying sizes. There were usually more nonrespirable CFUs than respirable CFUs identified between the two-stage and six-stage impactors and between the two cities. There were no culturable Gram-negative enteric bacteria isolated from the ambient aerosols. This is an important finding because it decreases the chance of enteric diseases being transported by bioaerosols in ambient air. Gram-negative enteric bioaerosols are very susceptible to ultraviolet light (Chang et al. 1985) and desiccation (Marthi et al. 1990; Purdy et al. 2004), and these factors probably contribute to their absence in ambient air.

The summary of the main findings of this paper is that feedyards probably increase the concentration of $PM_{2.5}$ particulates in and

around cities where they are located. This is of considerable importance because PM_{2.5} particulates are respirable.

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