BIOAEROSOLS GENERATED FROM BIOSOLIDS APPLIED FARM FIELDS IN WOOD COUNTY, OHIO

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ABSTRACT

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Recently there has been increased interest in using biosolids to fertilize farm fields due to an amendment to the United States Ocean Dumping Act (1988). The amendment imposes a ban on dumping waste material into the sea. Although much care has been taken to reduce its toxic and pathogen content, employees engaged in biosolids application in the farm fields and residents near the sludge-applied fields have reported health problems after its application. This research explores temporal variation in the quality of bioaerosols generated from class B biosolids applied to farm fields at different places around Bowling Green, Ohio.

Two types of air samplers, Microflow sampler (single stage sampler) and Andersen's six stage sampler were used to collect the bioaerosols. The combined data of both samplers were used to study the temporal variation of bioaerosols during and after class B biosolid application to farm fields.

All of the data show higher numbers of bacteria colonies collected from the downwind direction than from upwind. Compared to the data collected on the day of application, total bacteria, *Staphylococcus aureus*, and gram-negative bacteria were elevated 2 days after biosolids application. Levels decreased to control level 13 days after application, except for *Staphylococcus aureus*, which was highest 13 days after application. It can be concluded that pathogenically nontreated class B biosolids are capable of generating potential pathogens in the air. This increased content might be responsible for reported health problems in nearby residents during the post-application period.

Also there is a possibility that the finer particles, which constitute approximately 50% of the total bioaerosols generated from the fields, can be transported some distance away from the

class B biosolids-applied field. These finer particles containing pathogens might be responsible for health problems in residents a mile away from the field. However, further research is necessary to come to a definite conclusion in this regard. This research will increase awareness regarding the possible prohibition of class B biosolids in favor of class A biosolids, which are biologically treated sludge that contain comparatively fewer bacteria.

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INTRODUCTION

Purpose

Biosolids, commonly known as sewage sludge, are the solid or semisolid remnants of domestic sewage that has been produced during wastewater treatment. Waste materials that are produced in the wastewater treatment plant are disposed of in three ways: application to agricultural fields, landfilling, and incineration. Before the 1990s the most common methods for disposing of biosolids were land filling and incineration. However, the application of biosolids as fertilizer has become common since 1988, when an amendment to the United States (U.S.) Ocean Dumping Act imposed a ban on the dumping of sewage sludge and other kinds of waste materials into the open seas (Powell et al., 1998). Recent studies have determined that biosolids provide most of the nutrients required for plant growth (Urasa et al., 1999), which may be another cause of promoting the use of biosolids in farm fields.

In Europe and North America approximately 40% of the total biosolids produced in wastewater treatment plants (WWTP) are applied to farm fields, another 40% are taken to landfills, and the remaining 20% are incinerated (Parmar et al., 2001). Currently 50% of the total biosolids produced in the United States are used as fertilizer. In Ohio the total amount of sludge used in 2001 was 447,710 dry tons (Bowman, 2001). As much as 260,491 dry tons of biosolids were applied to the land, which was 58% of the total amount. The rest was disposed of in landfills (10%), incinerated (26%), or exported out of the state (6%). In the Northwest Ohio EPA district, as much as 92% (2001) and 93% (2002) of total biosolids were used for land application by local publicly owned treatment works (POTWs). The remaining sludge was used as landfill. In 2002 the total amount of biosolids produced in Ohio was around 419,335 dry tons, of which

59% was applied in the land, 28%incinerated, 9% used as landfill, and 4% exported out of the state (Ohio EPA, Sewage Sludge Management Program, 2002).

It has been reported that the workers and residents near the biosolids-applied farm fields complained about various health hazards after the application of biosolids (Burton et al., 1998). Therefore, it is important to know if biosolids application in the field generates bacteria into the air and, if so, how far the aerosols might transport. Sewage-sludge-related health complaints are most likely due to inhalation and ingestion of the aerosols released after its application. These aerosols could be responsible for different kinds of diseases, including respiratory problems (Lewis et al., 2002).

Biological aerosols are the biological particles in the air (including pathogens) that can be aerosolized by human or natural activity (Brooks et al., 2005). In this research an attempt has been made to explore the quality of the biological aerosol (or bioaerosol) generated from the biosolids-applied farm fields, in terms of total bacteria, *Staphylococcus aureus* (henceforth *S. aureus*) and gram-negative bacilli (bacillus: rod-shaped bacteria) that are potentially pathogenic. *S. aureus* is a gram-positive coccus (coccus: spherical-shaped bacteria) responsible for upper and lower respiratory tract infections, as well as pneumonia in acute cases. Some of the gram-negative organisms (such as coliforms, including *Escherichia coli*) are also potentially pathogenic and may cause diseases such as gastroenteritis and renal failure.

Soil Composition of Wood County

The natural aerosol content of a particular place depends on the nature and the aerial exposure (not covered by grass or other plants) of the soil of that area. The porosity and permeability of the soil have some effect on the aerosol content of an area. For instance, if the top soil is more permeable, water can flow downward in less time; consequently the sediment

dries quickly. Dry top soil containing microbes can easily be aerosolized. Most of the soils in the northwestern part of Ohio, including Wood County, originated from lacustrine sediments or from calcareous glacial till that were later reworked by lake water. Soil texture varies from fine clays to sands with a higher percentage of clay.

Approximately 400 soil series are found in Ohio (Beuerlein et al., 2004). The Aurand soil series covers most of the northwestern part of Ohio, including Wood County (United States Department of Agriculture, 2005). The Aurand series is characterized by moderate permeability in the upper part of the solum, and low permeability in the substratum. The solum is the upper and most weathered portion of the soil profile, which is plastic and sticky when wet, and hard when dry. The texture of the "A" soil horizon (0 to 11 inches) is characterized by a grayish to dark brown colored loam and a fine to medium granular structure with 2% rock fragments. It is slightly acidic (pH 6.1 to 6.5) with a moderately cemented iron and manganese concentration in the matrix. Generally it contains good amounts of phosphorous and potassium.

Climate

The climate of an area also affects the aerosol content. Temperature influences bioaerosol content because the quantity of bacteria can vary with temperature. Bacteria can thrive in almost any environment on the earth. On the basis of growing temperature, they can be classified into three broad categories. Psychrophiles are those that can grow at a temperature range of 0ºC to 20ºC, mesophiles grow in a temperature range of 20ºC to 40ºC, and thermophiles grows at 40ºC to 90ºC or higher. Most of the common pathogens are mesophiles and grow at an optimum temperature of 37ºC. Monthly mean temperature of Bowling Green, Ohio varies from - 5°C in the winter (December–January) to 23°C in the summer (August). Therefore, there is a possibility of finding considerably higher numbers of bacteria colonies in the summer.

The load of suspended particles in an area also depends on the wind speed, amount of precipitation, and snow fall. In general, wind speed is at maximum (approximately 18 km/hr) during the winter and at minimum (approximately 11km/hr) during summer. High amounts of precipitation and snowfall reduce the quantity of suspended particles in the air. Average annual snowfall at Toledo (32 Km NE of Bowling Green) is 94 cm. The maximum amount of snowfall, approximately 25 cm, occurs in December. Bowling Green, Ohio receives an average annual precipitation of 84 cm, as measured at the WWTP, Bowling Green, Ohio. The amount of precipitation is at its maximum (more than 9 cm per month) during the months of April through August, and at its minimum (less than 5 cm) during winter (December–January).

Biosolids Preparation Process

Biosolids are used as fertilizer in all of 50 states of the United States (Bowman, 2001). The federal biosolids rule contained in 40CFR, Part 503, administers the use or disposal of biosolids to protect both human and animal health near biosolids-applied farm fields (EPA, 1994). Biosolids are classified on the basis of three parameters: level of pollutant metals, availability of pathogens (for example, Salmonella bacteria, enteric viruses, and viable helminthes ova), and the degree of vector attraction (such as mosquito, rodents, flies, and birds). According to Part 503 of the regulation there are two classes of pathogen-reduced biosolids: class A and class B. Class A biosolids are almost pathogen free and comply with the following standard: less than 1000 fecal coliforms g^{-1} MPN (most probable number); less than 3 *Salmonella* per 4 g total solids (TS); less than 1 plaque-forming viral unit per 4 g TS; and less than 1 viable helminth per 4 g TS. Class B biosolids, on the other hand contain pathogens that are significantly reduced, but still present.

Biosolids are produced at the wastewater treatment plant during the treatment of wastewater collected from domestic, commercial, and industrial sources (EPA, 1999). The wastewater treatment plant at Bowling Green has been in operation since 1982. It serves the needs of both the city of Bowling Green and the village of Portage. Treatments are accomplished by primary, secondary, and tertiary (advanced) processes. At each interval the percentage of solid and liquid material in the biosolids varies. Most of the biosolids are subjected to further treatment for pathogen reduction (e.g. stabilization, dewatering, and others) to meet the federal regulatory requirements for the protection of human and animal health.

The processes used for class B biosolids are alkaline stabilization, anaerobic digestion, aerobic digestion, composting, and heat drying. These processes can significantly reduce pathogen content but not up to the level recommended in 40CFR Part 503. Therefore, restrictions are imposed for using class B biosolids. The processes applied for class A include a high pH application, composting, heat drying (palletizing), heat treatment, thermophillic aerobic digestion, beta ray irradiation, and gamma ray irradiation. These processes make the biosolids substantially pathogen free; therefore, there are no such restrictions imposed for using class A biosolids.

Alkaline stabilization reduces pathogen content, removes odor, and reduces the acidic nature of the raw sludge. Anaerobic digestion is a process, where anaerobic microorganisms are used to digest the biosolids in a closed environment, which reduces the pathogenic organisms as well as the mass of the sludge. Aerobic digestion basically does the same, except in an oxygenated environment, and the microorganisms used are aerobic in nature. During composting, the biosolids are degraded into humus-like material in a high-temperature (55˚C - 60˚C) controlled oxygen and moisture condition. In heat drying, active and passive driers are

used, and the mass of the biosolids is significantly reduced. Dewatering decreases the volume of biosolids by reducing the water content in it. Normally dewatering is conducted before composting, incineration, or other processes (EPA, 1999).

Burton et al. (1998) conducted research on airborne bacteria around LeSourdsville, Ohio. During the land application of class B biosolids, they observed that the bacterial concentration of the air ranged from 694 cfu/cubic meter with a geometric standard deviation (g.s.d.) of 1.5 cfu/cubic meter to 2,356 cfu/cubic meter (with a g.s.d. of 1.3 cfu/cubic meter). Bacillus, *Flavimonous sp., Mycobacterium sp., Pseudomonas sp., Staphylococcus sp.,* and *Streptococcus sp.* are the common bacteria found in air. Studies show that the workers and the residents near the biosolids-applied fields have complained about different kinds of diseases (Gregersen et al., 1999; Ranalli et al., 2000). Four out of five people interviewed reported repeated or intermittent episodes of gastrointestinal symptoms, including diarrhea and abdominal cramping (Burton et al., 1998). One reported abdominal cramps after working in the farm field. In addition, biosolids contain ammonia, which has a strong odor (EPA, 2003. "Biosolids, Frequently asked questions") and causes health problems such as nausea, headache, and breathing difficulty.

In addition to the pathogenic organisms listed previously, biosolids contain nitrogen, phosphorus, potassium, and heavy metals such as cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), and lead (Pb), of which cadmium, copper, and lead are toxic (Logan et al., 2004). Biosolids also attract vectors such as rodents, flies, and mosquitoes, each of which can cause a number of diseases.

Class B biosolids are the most commonly used biosolids for agriculture in the state of Ohio. Many agricultural farms (up to 10% of the total) in Wood County used class B biosolids in the past; however, the Bowling Green WWTP started producing class A biosolids in June 2005.

In addition to sludge-handling employees, a study among 48 residents from North America and Canada (Lewis et al., 2002) found that the dwellers near the biosolids-applied farm fields also reported different types of symptoms, including coughs; burning eyes, throat, or lungs; headaches; trouble breathing, and others (Table 1). According to the report, the symptoms developed between an hour to a month after application of class B biosolids. Medical reports revealed that most of the infections were caused by *S. aureus,* which could be transmitted by air.

Table1. *An Investigation of the Symptoms of Farmers and Residents around the Biosolids Applied Farm Fields in Different parts of the Northern United States and Canada*

Site	Distance (Km)	Age/sex	Smoker	Symptoms
Green Land, NH	0.2	53, F	N	C, Cn, Db, H, N, D
Green Land, NH	< 0.2	26, M	N	C, Cn, H, F
Waynesville, OH	0.2	60, F	N	C, Be, Bt, Bal, Db, S, R
Waynesville, OH	0.1	78, M	N	C, Db, S
Cedarville, ON	0.2	51, M	N	Be, Bt, Bal
Robsoria, PA	0.2	15, F	N	C, Cn, Db, P, H, Fv, F, Si
Osceola Mills, PA	0.8	11, F	N	Be, Bt, Cn, H, Si, N, F, Ft

Note. Abbreviations: (Be) burning eyes, (Bl) burning lungs, (Bt) burning throat, (C) cough, (Cn) congestion, (D) diarrhea, (Db) difficulty breathing, (F) flu, (Ft) fatigue, (Fv) fever, (H) headache, (N) nausea/vomiting, (P) pneumonia, (R) skin rash, (S) sinusitis, and (Si) staphylococcal infection in human respiratory system. Source: Lewis et al., 2002.

Bioaerosols can be transported by wind for a long distance. Brandi et al. (2000) measured the growth and extent of microbes in different air velocity conditions at different times after sludge application operations were started. They conducted the experiment around two wastewater treatment plants along the northern Adriatic Sea coast. The experiment was also conducted during different seasons over one year (Table 2). A surface air system (SAS) sampler was used for sample collection, and the results show no bacteria before and a day after the plants had started the operation. However, there was an exponential increase in bacterial content of air after 3 days, 12 days, and 25 days after the operation was started. The common microorganisms

that were found around the treatment plants after a long treatment operation were coliforms, *Escherichia coli,* and staphylococcus.

Staphylococci are the gram-positive coccus bacteria that appear like a cluster of spherical cells under a microscope. They are available in abundance in the human nose and skin and act like a potential pathogen. However, it is difficult to conclude at which point they are altered to become a pathogen. Although more than 20 species of *Staphylococcus* are found in nature,

Table 2. *Variation in Mean (mean of three experiments) Microorganism Concentration in Different Seasons Collected in the Surface Air System Sampler, Expressed in Colony Forming Units (cfu)/Cubic Meter in Como, Italy*

		Season		
Micro-organism	Positions	Winter	Spring	Summer
Total bacterial count	2 m upwind	8.9	5.5	66.6
	2 m downwind	298.8	11.1	222.0
	10 m downwind	170.5	11.1	105.4
Total fungal count	2 m upwind	27.5	38.8	92.0
	2 m downwind	147.2	38.8	190.0
	10 m downwind	62.3	38.8	106.0
Total Coliforms	2 m upwind	nd	nd	nd
	2 m downwind	nd	nd	nd
	10 m downwind	1.3*	nd	nd
Enterococci	2 m upwind	nd	nd	nd
	2 m downwind	nd	2.7*	nd
	10 m downwind	nd	nd	nd
Escherichia coli	2 m upwind	nd	nd	nd
	2 m downwind	nd	nd	nd
	10 m downwind	nd	nd	nd
Staphylococci	2 m upwind	nd	nd	nd
	2 m downwind	12.4	20.8	24.9
	10 m downwind	6.9	8.3	11.1

Note. nd = not detected. * Value of a single sampling. From Brandi et al., 2000.

only *S. aureus* and *Staphylococcus epidermidis* (*S. epidermidis*) are significant in their interactions with humans (Todder, 2005). *S. aureus* mainly inhabits the nasal passages; however, it can often be found in other human organs. On the other hand, *S. epidermidis* is found in the

skin. Various types of suppurative (pus-forming) infections in humans can be caused by staphylococci. Staphylococci are also responsible for superficial skin lesions, such as boils, styes, and furunculous; more serious infections, such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis. Staphylococcal food poisoning is the second most reported infection in the United States; however, it is to be noted that the most food poisoning cases are not reported (Alcamo, 2001).

Escherichia coli (*E. coli*) is a type of gram-negative anaerobic rod and a commonly found normal gut flora. Like staphylococcus, it can also be potentially pathogenic and it is difficult to determine at which point it establishes a pathogenic relationship (Norton, 1981). *E. coli* is responsible for enteric diseases like diarrhea, dysentery, and other types of diseases, such as kidney infections and enterocolitis. Other gram-negative bacteria can also be considered opportunistically pathogenic.

MATERIALS AND METHODS

Sampling Location

Samples were collected from two types of areas: George Riker's field, northeast of Bowling Green, Ohio, where class B biosolids have been applied; and the football grounds adjacent to the Doyt Perry Stadium, Bowling Green, Ohio (henceforth, football stadium), which is 1.8 kms southwest of Riker's field, where biosolids have not been applied (Figure 1). At George Riker's farm field, samples were collected on,on the day of biosolids-application (Figure 2), four days before, two days after and thirteen days after the application.

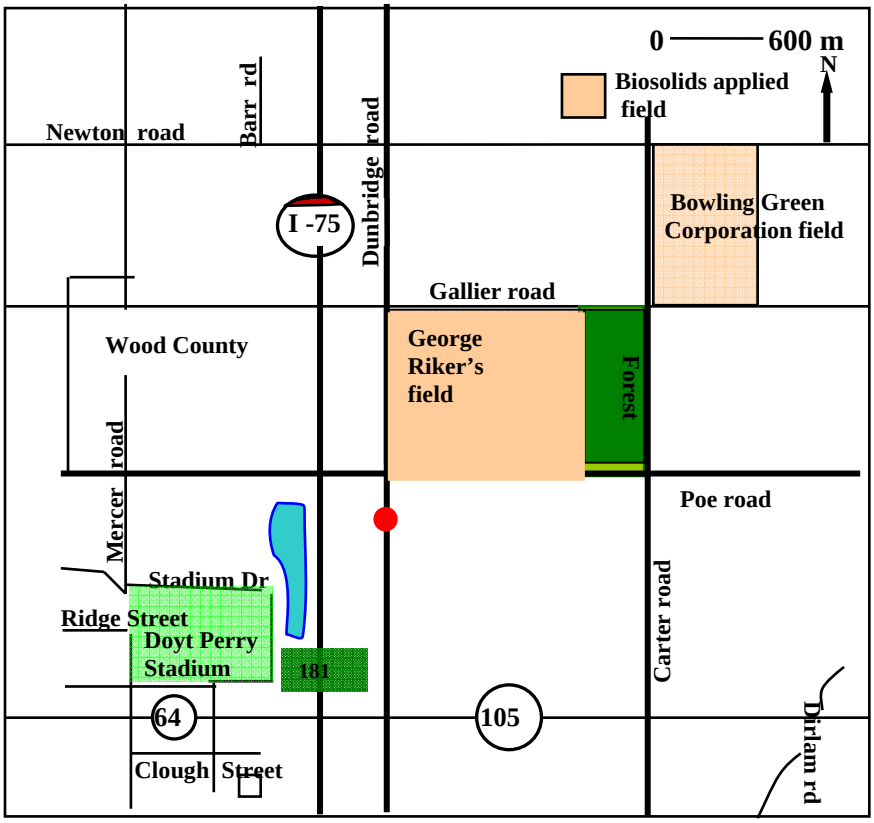

Figure 1. Locations of George Riker's farm field, Bowling Green Corporation farm field, Bowling Green Sewage Treatment Plant, and The Football field adjacent to Doyt Perry Stadium, Bowling Green, Ohio are shown. The bioaerosol samples were collected from the eastern part of Riker's field.

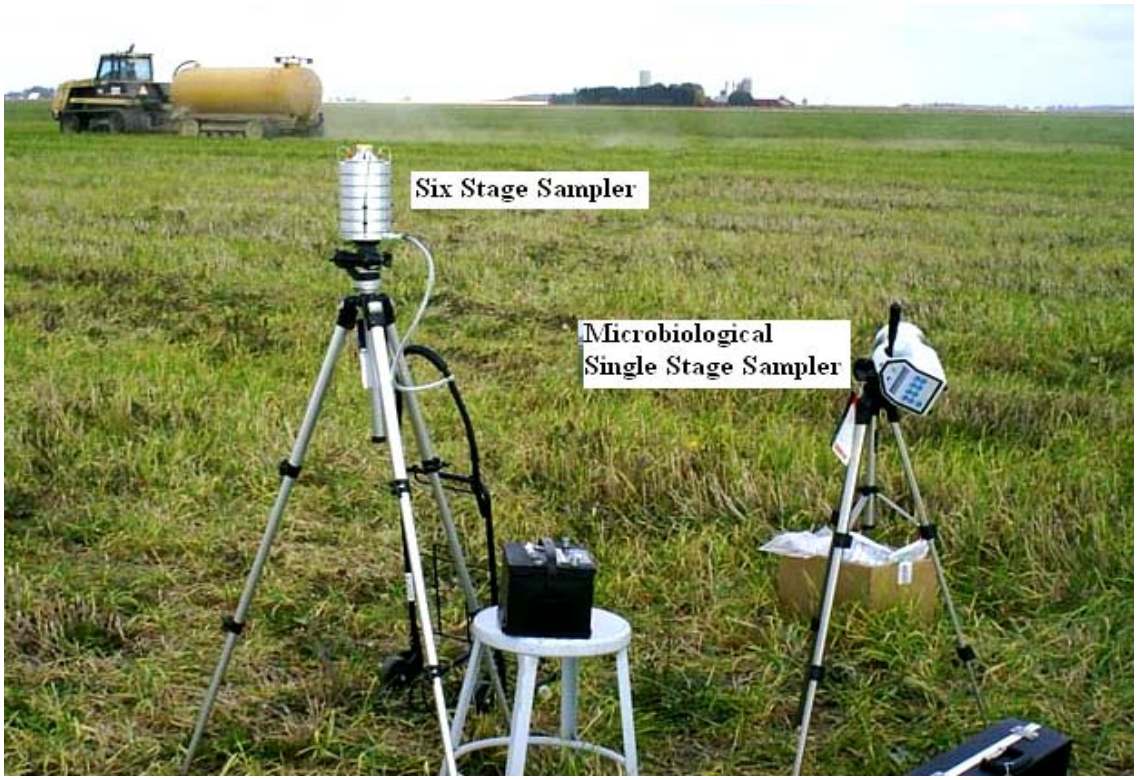

Figure 2. Samples were collected during biosolids application.

The purpose of collecting the data from the football stadium was to explore any correlation with respect to variation of the bioaerosols in an area 1.8 km (1.1 miles) away from the biosolids-applied field. A third group of samples was also collected from the class B biosolids-applied Bowling Green Corporation field, a month after its application. However, the variables such as temperature, wind speed and humidity during the sample collection were different for Riker's field and the Bowling Green Corporation field. Therefore, Bowling Green Corporation field-samples were ultimately ignored and the Riker's field-samples were only used for temporal variation study of bioaerosols. Some samples were collected at Bowling Green WWTP at 2 meters upwind and 2 meters downwind of the processed biosolids reservoir. It should be noted that the football stadium is located only about a quarter mile WSW of the WWTP (across I-75 from the plant). Therefore, it can be concluded that a NE wind towards the Football stadium can carry the bioaerosols generated from both Riker's field and WWTP.

Selected data from the entire samples was later used to establish a correlation between the two samplers (described in next section) used for data collection.

Table 3. *Number of Samples Collected*

		Location					
		Riker's Farm		BG WWTP	BG Corp. Field		
Date	Football Stadium	Up-wind	Down-wind			Sampler	Biosolids application
9/30/04*	N	N	Y	N	N	1,2	Y
10/7/04	Y	Y	Y	N	N	2	N
10/11/04	Y	Y	Y	N	N	1,2	Y
11/10/04	N	Y	Y	N	N	1	Y
11/12/04	Y	Y	Y	N	N	1,2	N
11/23/04	Y	Y	Y	N	N	2	N
03/30/05	Y	Y	Y	Y	Y	1,2	N

Note. 9/30/04* Data not used due to contamination; Y- day of biosolids application; N- day of non-application; 1 = Andersen's six stage sampler; 2 = Microflow sampler; BG field = Bowling Green Corporation field.

Air Sampler

Two types of air samplers were used to accomplish the sampling process: a single-stage sampler called Microflow, and Anderson's Six Stage Viable Impactor (Henceforth, Andersen's sampler). Both are impactor type air samplers. The air containing the bioaerosols is drawn inside the sampler, which impacts on a bacteria culturing medium. The medium is placed inside the sampler. The Microflow sampler is a single-stage sampler (Figure 3) and draws air through the perforated head at a constant rate. The flow rate can be changed manually. The processor for this sampler is able to store sampling data (up to 99 samples) including time, date, total amount of air sampled (in liters), and flow rate. The Microflow sampler was placed at a height of approximately 1.2 meters. The flow rate was fixed at a rate of 30 liters per minute, and sampling time varied from 15 to 20 minutes. Plastic petri dishes (90 mm diameter) with selected media were used inside the sampler. Both samplers were connected with air pumps. The Microflow sampler has an inbuilt air pump, whereas Andersen's sampler is attached to an external air pump.

Figure 3. The Microflow, single-stage sampler and perforated head are shown.

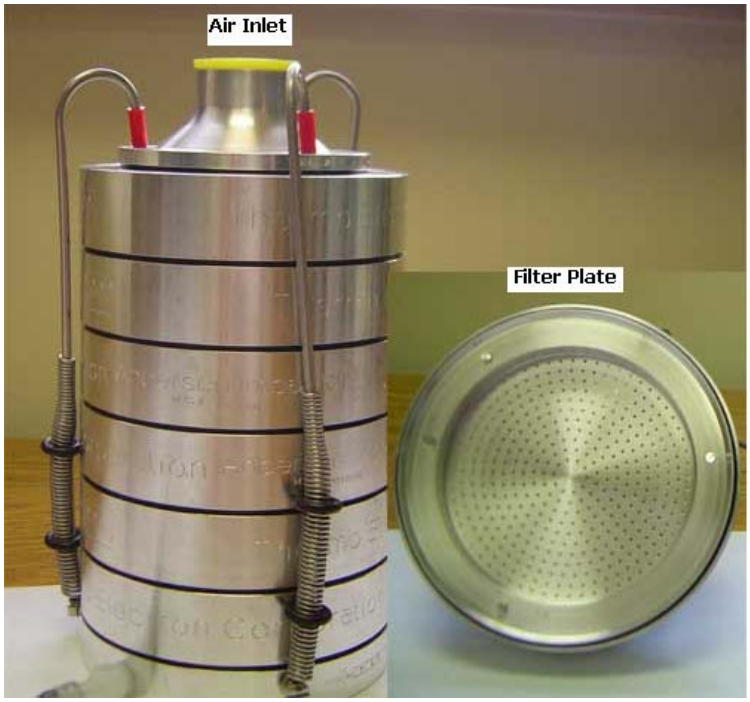

Figure 4. The Anderson's six-stage sampler and filter plate of stage one are shown.

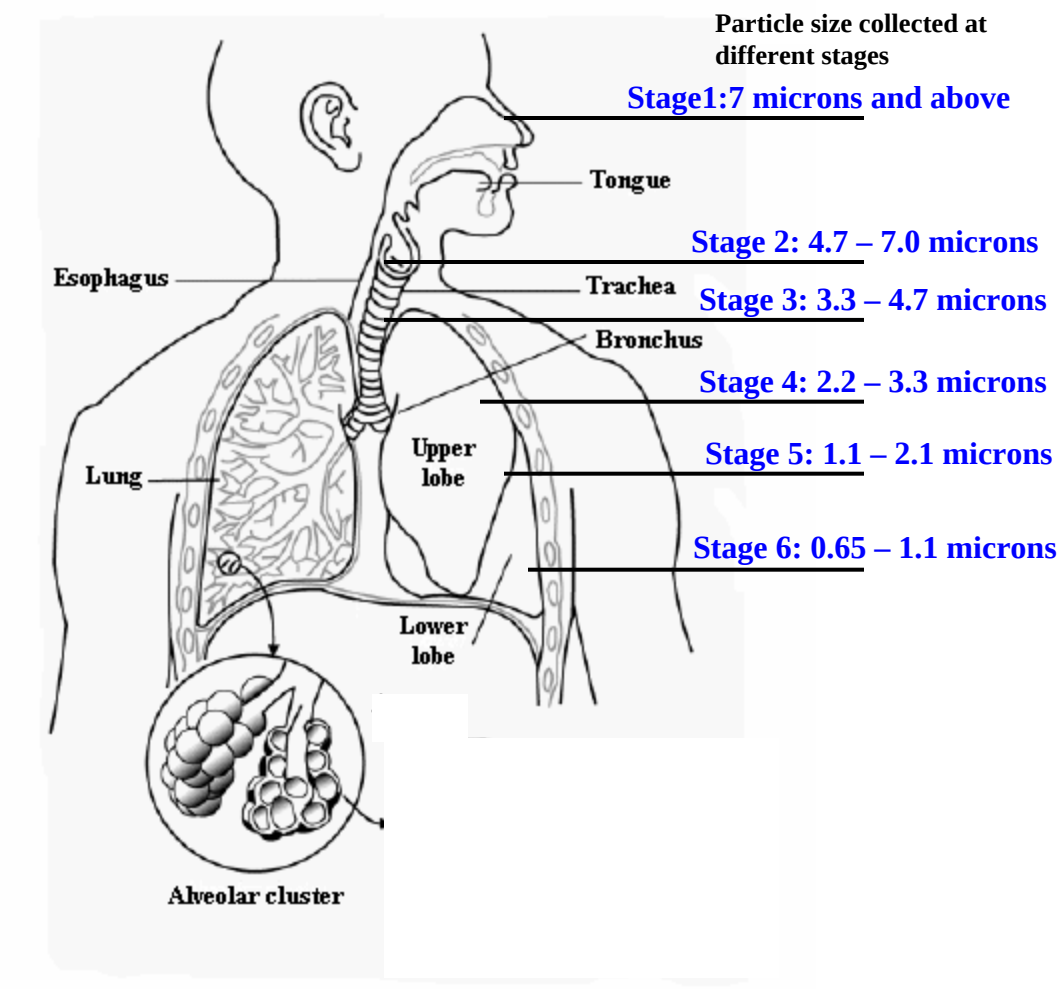

Figure 5. Andersen's sampler, a comparison with human respiratory tract, with diagram of human respiratory system from http://www.asosh.org/Programmes/SORDSA/_derived/Crystalline_silica.htm_txt_Lung.gif

The Andersen's sampler is a six stage sampler (Figure 4), and each stage is attached with a filter comprised of 400 orifices. The diameter of the orifices ranges from 1.2 mm at the first stage at the top to 0.25 mm on the sixth plate at the bottom. However, the size of each orifice is constant for a particular stage. This particular sampler simulates the human respiratory system (Figure 5) with a notion that the human respiratory tract is an aerodynamic classifying system of airborne particles. This sampler was used to estimate the extent to which pathogens can penetrate to the upper and lower respiratory tracts. A constant flow rate of 28.3 liters per minute was

maintained for this sampler, which is a fixed rate with respect to its configuration. This sampler was placed at a height of 1.5 meters, which is the average height of a human nose from the ground. The sampling time varied from 15 to 20 minutes. There is no particular standard for sampling time, and it should be noted that sampling for more than 30 minutes may not only dehydrate the media, but it can also damage the already collected particles (Thermo Electron Corporation environmental instruments, 2003).

An experiment performed by Andersen (1958) showed that plastic petri dishes collect 20% less counts than glass dishes. The static charges generated from the plastic petri dishes inhibit the aerosol particles at the exterior of the plates. The glass petri dishes, which have a specific diameter and height and come with the sampler, are the only recommended media plates for this purpose. However, because of the convenience of using plastic petri dishes, they were used in this study. Dr George Bullerjahn from the Department of Biology, Bowling Green State University, and Dr. Michael Bisesi from Medical University of Ohio also supported the use of plastic petri dishes for the following reasons. The glass petri dishes provided with the Andersen's sampler have aluminum lids, which make it difficult to identify the colonies in the laboratory. On the other hand, the plastic petri dishes have transparent plastic covers, and colonies can easily be identified from both sides without opening the lid; consequently, any possible post sampling contamination can be avoided. Also, plastic dishes are light in weight, and, easy to carry to the field. Sterilization is an important step for any biological instrument. For glass petri dishes, sterilization is necessary prior to each use, whereas plastic Petri dishes are available in inexpensive sterile packs that save time. For sampling, 100 mm x 15 mm plastic petri dishes were used with Andersen's sampler, and this researcher verified that they were approximately the same size as the glass petri dishes provided.

Andersen's sampler needs to be calibrated during a change in temperature and pressure. Because the size fraction of each stage is controlled by the orifice velocities, it is important to maintain the volume of air as 1 ACFM (actual cubic feet per minute, which is equal to 28.3 liters per minute). The sampler was calibrated at an ambient temperature and pressure in Atlanta, Georgia before its shipment. A secondary gas flow meter, which is a secondary tool for flow measurement, came along with the sampler and can be used to measure the amount of air drawn inside before each use.

Media Preparation

Nutrient Agar, which is a general media supporting the growth of nonfastidious organisms, was mainly used in both of the samplers during sample collection. However, some special types of media, such as Mannitol Salt Agar, Potato Dextrose Agar, and LB Agar, were sometimes used for collecting particular types of bacteria. Mannitol Salt and Sheep Blood Agar, which are special types of media for maintaining and propagating gram-positive coccus bacteria, were used for subculturing and identifying staphylococcus (mainly *S. aureus*). MacConkey and LB Agar were used for identification of *E. coli* and other gram-negative bacillus (rod-shaped) bacteria. All of the previously mentioned media are commercially available in powdered form. Containers of 100 gm or 500 gm or more can be purchased through the reference number provided in the Difco Biological Manual. All of the media were suspended in water separately, autoclaved for 15 to 30 minutes at 121°C, then poured into the petri dishes. The recipe for each particular medium is provided on the label attached to the container. Prior to sampling, media plates were chosen randomly and put into an incubator for 24 hours to check for any possible contamination; the contaminated petri dishes were discarded.

Precautionary measures that should be taken by persons dealing with collection and analysis of biosolids samples are described in 40 CFR Part 503 (EPA, 2003). However, there are no such recommendations for workers engaged in collection of aerosols around biosolids-applied fields. It was assumed that aerosols above biosolids-applied farm field areas would contain pathogenic organisms, and therefore the necessary safety measures were taken. The safety practices during collection and laboratory analysis were as follows:

1. Gloves should be used during collection and analysis of the samples.
2. Hands should be washed frequently and always before eating, smoking, and other activities.
3. Photocell-activated or foot-activated hand washing stations are desirable to reduce spreading of contamination to others.
4. Workers should be trained not to touch their lips or eyes during collection.
5. Mouth pipetting in the laboratory should be forbidden.
6. Workers should have a good immunization history (such as tetanus, hepatitis A and B).

At the end of the sampling process, all of the collected petri dishes were sealed with para films to avoid any possible contamination and were brought to the laboratory within an hour of collection, where they were allowed to incubate upside down for 24 to 48 hours at 37°C. Afterward, the number of colonies was calculated by using a colony counter and expressed in colony forming units per cubic meter (cfu/cubic meter) of air. The temperature and wind speed data during sampling were collected from the weather station at the Bowling Green WWTP.

Identification of Bacteria

The primary objective of this experiment was to count the total number of bacterial colonies, total number of staphylococcus, and total number of gram-negative bacillus. Almost all

of the staphylococcus (80%) colonies were identified as *S. aureus*. Primarily the colonies were identified by their morphology, followed by microscopic identification and subculturing into specialized media.

Controversies are common regarding the identification of *S. aureus*. Russin et al. (2003) have criticized the use of Mannitol Salt, as described in previous literature, as the only media for identifying *S. aureus.* Therefore, in this thesis research a number of experiments, such as gram staining, subculturing into various types of specialized media (Figure 6), and the catalase test have been employed to identify *S. aureus*. This research did not require identification of staphylococci at the genetic level; therefore, the Polymerase Chain Reaction Method was not applied. The researcher thinks that the conventional techniques, as described in the following, are sufficient to identify the total number of viable *S. aureus*.

Colonies that had white or yellow pin head morphology on Nutrient Agar and yellowish orange appearance in Mannitol Salt Agar were considered staphylococcus. Thereafter, some of the colonies from the original samples were chosen randomly for further investigation. Usually the first test performed for the identification of bacteria is gram staining, where the primary stain used is crystal violet. The microorganisms that retain the crystal violet-iodine complex appear purple under microscopic examination and are classified as gram positive (Figure 7). The other microorganisms that are not purple are referred to as gram negative (Figure 8) and appear red or pink due to the application of safranin. Gram staining is based on the ability of the bacteria cell wall to retain the crystal violet dye during solvent treatment. After gram staining, the samples are ready to be identified under a microscope, starting with at least 40x magnifications.

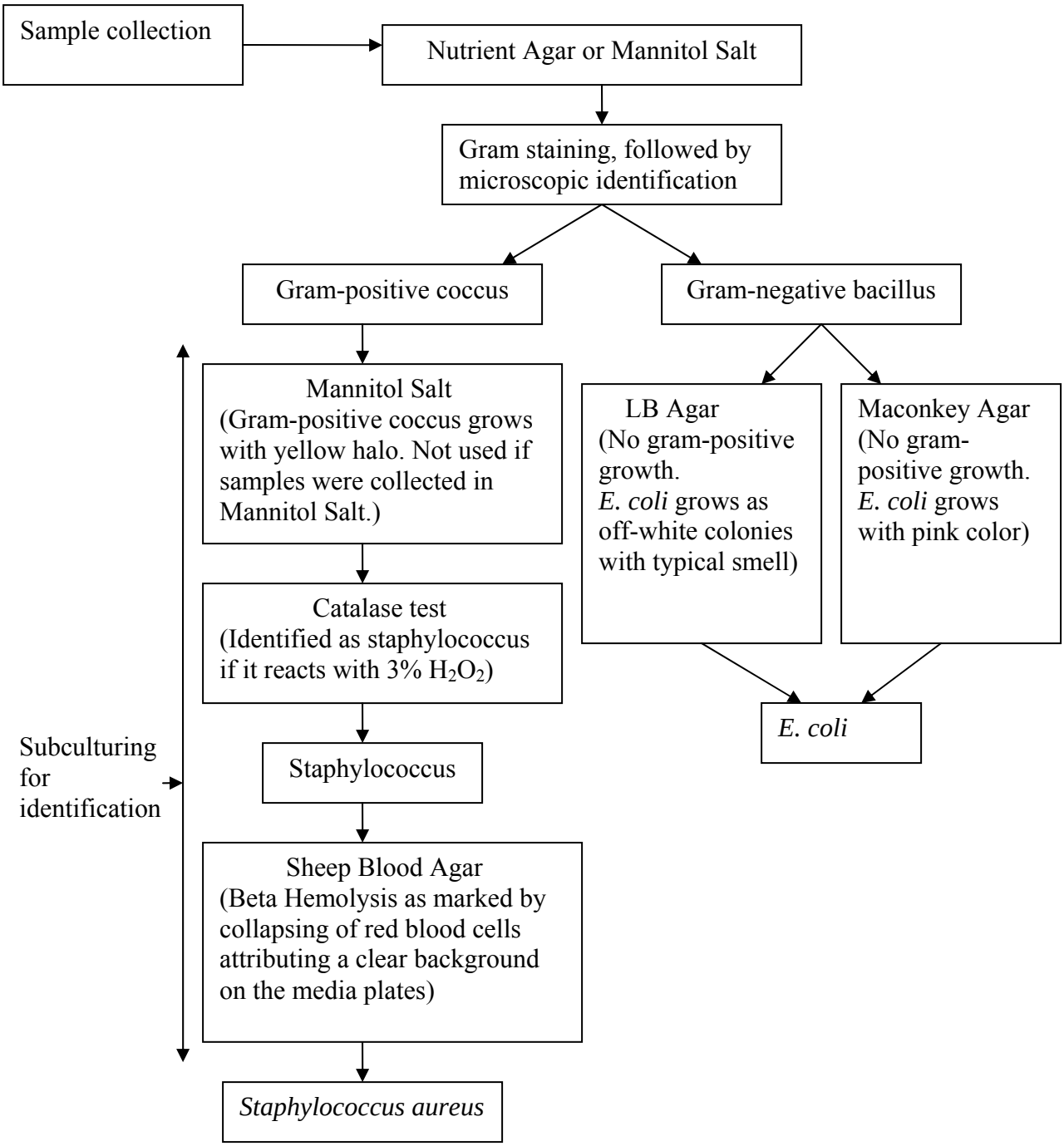

Figure 6. Measurement and identification protocol for *S. aureus* and gram negative bacteria.

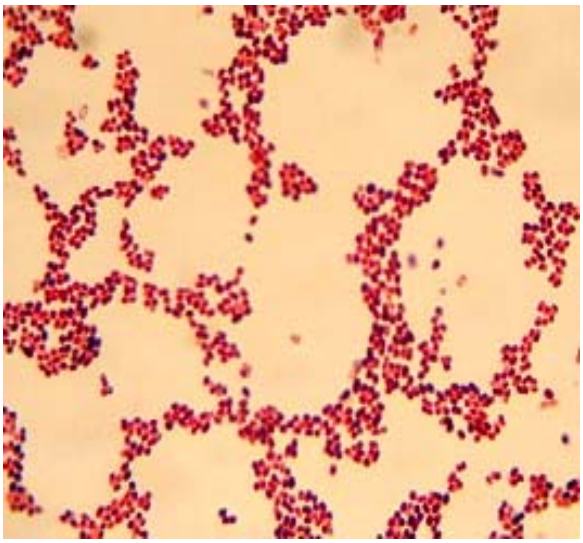

Figure 7. Gram-positive coccus *Staphylococcus aureus.* Magnification: 1000x

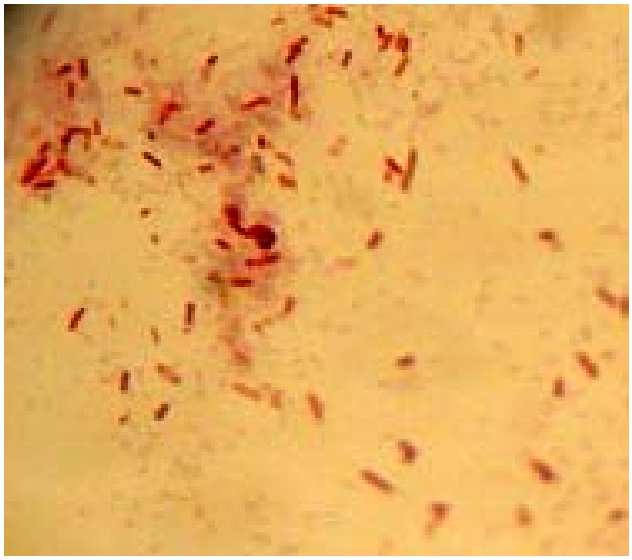

Figure 8. Gram-negative bacillus *Escherichia coli*. Magnification: 1000x

After microscopic identification, staphylococcus was identified by subculturing into specialized media. Staphylococcus grows in Mannitol Salt with a yellow halo; however, a streptococcus (another gram-positive coccus) can also display a yellow halo in Mannitol Salt. A catalase test was performed to differentiate staphylococcus from streptococcus. When staphylococcus was added to 3% H_2O_2, oxygen gas evolved, which is called catalase positive, whereas, streptococcus shows catalase negative (no gas bubbles).

Staphylococcus + $H_2O_2 \longrightarrow O_2\uparrow$ (Bubbles = Catalase positive)

Streptoacoccus + $H_2O_2 \longrightarrow$ (No Bubbles = Catalase negative)

Thereafter, a third test was carried out using Sheep Blood Agar, where *S. aureus* shows beta hemolysis, characterized by collapsing of red blood cells as evidenced by a clear background on the media plates (Figure 9).

E. coli is a type of gram-negative bacillus and is widely used as an indicator of the water quality in terms of enteric (gastro-intestinal) diseases. Several types of specialized media can

also be used to identify *E. coli* and other gram-negative coliforms that are opportunistically pathogenic and responsible for enteric diseases. However, the identification process was limited by only looking into the total gram-negative bacillus bacteria. The colonies were gram stained and identified under a microscope (Figure 6), followed by subculturing into specialized media such as LB Agar, where they grow as an off-white colony and release a typical smell. Gram-negative rods grow in MacConkey Agar with red color that is due to a change in the pH of the medium, whereas growth of gram-positive bacillus is inhibited in this medium.

A genetic correlation between the bacteria in biosolids-applied soil with that in the bioaerosols could provide a stronger evidence that the bioaerosols were originated from the biosolids applied field. But no soil samples were available to carry the research in this aspect. However, analysis of Variance (ANOVA) was carried out with three different types of samples such as: total bacteria, *S. aureus* and gram negative bacteria collected in five different dates. This analysis gives an idea whether those samples were originated from same population. Statistical software MINITAB 14 was used to accomplish this statistical analysis. In addition to this, EXCEL was used to make a correlation study between the bioaerosols with the temperature and wind speed for five different days of sampling. A correlation study gives a better understanding of the control of temperature and wind speed over biosolid contamination into the air. Also, EXCEL was used to plot a number of graphs to accomplish the analysis of temporal variation of bioaerosols at George Riker's field.

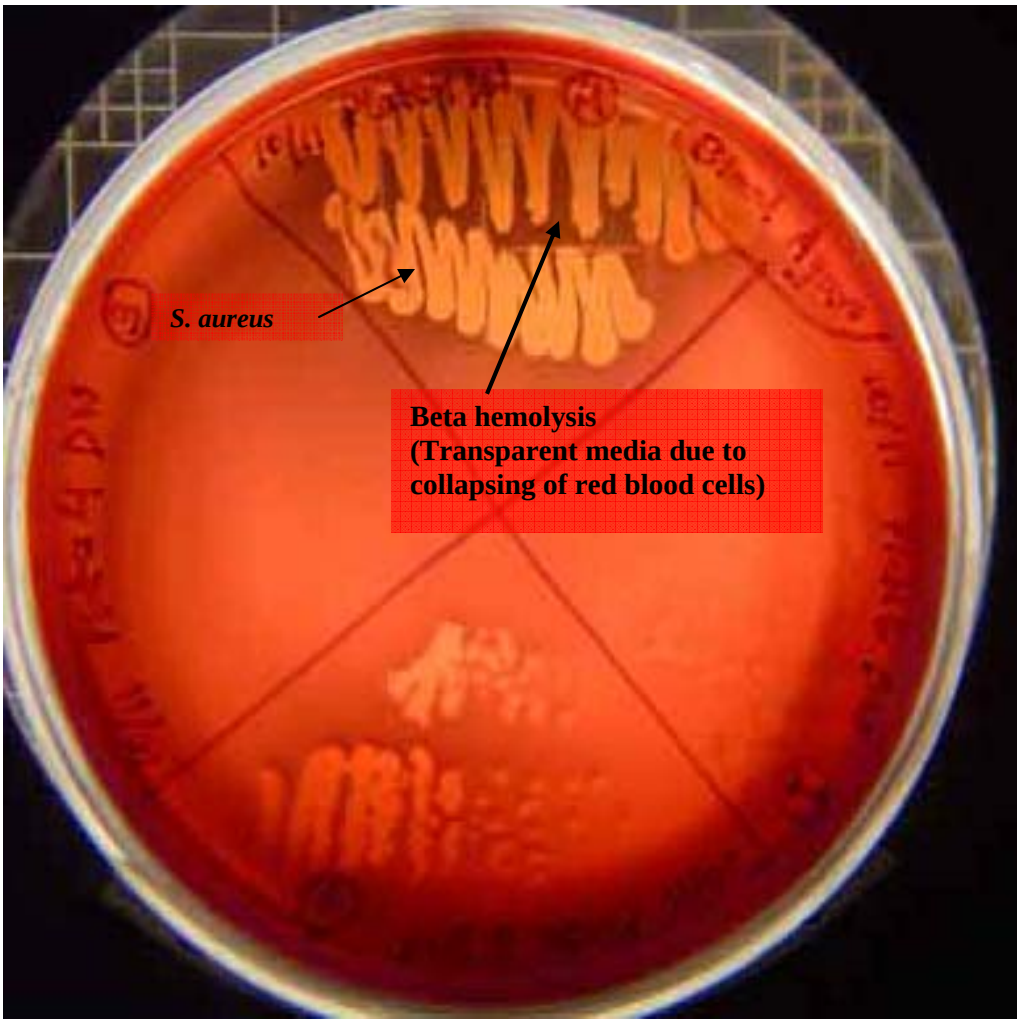

Figure 9. Arrow mark shows beta hemolysis by *S. aureus* in a Sheep Blood Agar medium. Beta hemolysis is characterized by the collapsing of red blood cells attributing a transparent background in between two serpentine shaped *Staphylococcus aureus* growths. This particular sample, as shown in the topmost quadrants, was taken from the 4th plate of Andersen's Sampler collected during biosolids application into the Riker's field (October 11, 2004), from the downwind direction.

RESULTS AND DISCUSSION

A list of figures (AI-1 to AI-25) in Appendix I show the nature of bioaerosols collected on different dates from George Riker's field, Bowling Green Corporation field, and the Bowling Green Sewage Treatment Plant. In each of the figures (except AI-6 to AI-9) there is an attempt to compare the data with that measured from the football stadium collected on the same day. This comparison is attempted in order to understand the variation of bioaerosols at a distance of 1.8 km from the biosolids-applied field. For a detailed analysis of the data collected by the Andersen's sampler, the number of colonies calculated in the first and the second plates were added together, as were the third and fourth plates and the fifth and sixth plates.

Figure AI-1 shows the data collected 4 days before application (10/07/2004). Figures AI-2 through AI-9 show the data collected on two different dates of biosolids application (10/11/2004 and 11/10/2004). Figures AI-10 through AI-14 show the data collected 2 days after its application (11/12/2004). Figure AI-15 shows the data taken 13 days after (11/23/2004) biosolids application. All of these samples (AI-1 to AI-15) were collected from George Riker's field, Bowling Green, Ohio and used for studying temporal variation of bioaerosols.

Another group of bioaerosol samples were taken from the Bowling Green Corporation Field during early summer 2005 (03/30/2005), which was a month after class B biosolids application (Figures AI-16 through AI-20) in that particular field. However, no samples were collected from George Riker's field at that time, because the application of class B biosolids had been stopped there. Samples were also collected at the Bowling Green WWTP on the same day (Figures AI-21 through AI-25) to get an idea of the possible impact of bioaerosols generated from the WWTP. This was the last month before the Bowling Green WWTP was switched from

class B to class A biosolids production. Seven chosen samples from this entire collection were used for a correlation study of the two samplers used in this research.

All the results show that for the same time of sampling, the Andersen's sampler collected an attenuated record of the total number of colonies in comparison to that of the Microflow sampler. Because of this discrepancy, it was necessary to determine the correlation between the data collected in a single plate of the Microflow sampler and all of Anderson's six plates added together. A linear relationship was found to exist (Figure10) between the total numbers of colonies collected by the two samplers. This equation was used to change all the data collected by the Microflow sampler to make the numbers equivalent to that collected by all six stages of Andersen's sampler together. This will be referred to as "sampler normalization" in this research. The R^2 value for the best fit straight line is 0.7, which illustrates a linear correlation between the total numbers of colonies collected by the two samplers. The R^2 value varies from 0 to 1 and the closer the value is to 1, the better the fit is. The root mean square value (Table 4), which is a measure of deviation of the predicted value from actual, is approximately 30 cfu /cubic meter. Following is the above stated equation:

$y = 0.6\,x - 6$ Equation (1)

where y = Estimate of the total number of bacteria colonies collected in all plates of Andersen's sampler.

x = Total number of bacterial colonies collected in Microflow sampler.

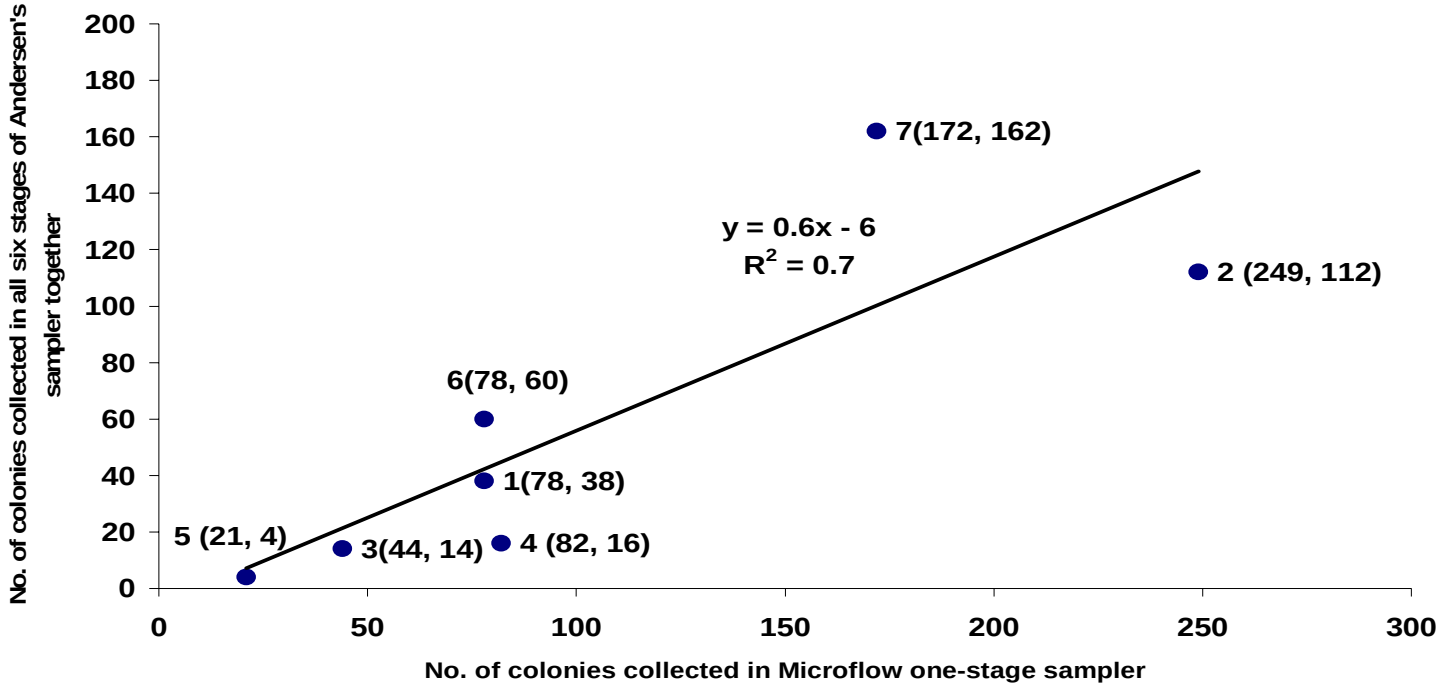

Figure 10. This figure shows the correlation between the data collected by all six stages of Andersen's sampler and the data collected by the Microflow sampler. Samples those are used for this correlation study, were collected from George Riker's field (point 2) on 11/12/04 (NE-NNE wind, 6.1ºC noon temperature); the football stadium (points 1 and 5) on 11/12/04 and 03/30/04 (SE wind, 19.4ºC noon temperature); Bowling Green WWTP (points 6 and 7) and Bowling Green Corporation field (points 3 and 4) Ohio, both on 03/30/04.It should be noted that the data collected in Microflow sampler was always higher (plotted along *y-axis*) than that collected in Andersen's sampler (plotted along *x-axis*).

Table 4. *Number of Total Bacterial Colonies Collected in Microflow sampler and Andersen's six stage sampler, and the Root Mean Square Value for the Best Fit Straight Line in Figure 10*

Location	Date		Microflow	All six (actual)	All six (predicted)	(actual-predicted)^2	Sum/n*	RMS
Field adjacent to Football stadium	11/12/04		78	38	42.32	18.67	901.72	30.03
Riker's farm	11/12/04	Up wind	249	112	147.78	1279.99		
		Up wind	44	14	21.35	54.07		
BG Field	03/30/05	Down wind	82	16	44.79	828.75		
Field adjacent to Football stadium	03/30/05		21	4	7.17	10.05		
BG Treatment Plant	03/30/05	Up wind	78	60	42.32	312.54		
		Down wind	172	162	100.29	3807.99		
		Mean		58				
		Median		38				
		Standard deviation		58.81				

Note. All six (Actual) = Total Bacteria (colonies per cubic meter) collected in all six plates of Andersen's sampler. All six (predicted) = Values calculated from the equation 1 (page 25) using the actual values. n* = Total number of samples; RMS = Root Mean Square

Glass petri dishes collect 20% higher number of colonies as compared to plastic petri dishes. Therefore, another correction was made (Equation 2) to normalize the entire data collected by plastic petri dishes to make it comparable with the data collected by glass petri dishes.

$$y = (100/ 80)\, x \qquad \text{Equation (2)}$$

where, y = total number of cfu/cubic meter collected in glass petri dishes, and x = total number of cfu/cubic meter collected in plastic petri dishes

After all these corrections were made, it was observed that higher numbers of colonies were always found from the downwind direction in comparison to upwind. It is assumed that the excess number of colonies in the downwind direction (i.e., downwind minus upwind cfu/cubic meter) was coming only from the biosolids-applied field and no other sources. These excess values (Table 5) have been used to study the temporal variation of bioaerosols with respect to pre and post-application of biosolids in Riker's field (Figures 11 to 14). Figure 11 clearly depicts that bioaerosols content was at its minimum, four days prior to its application; and was maximum two days after it was applied to Riker's field. Another bioaerosols-variation study has been made (Figures 15 to 17) with the data collected from the football stadium, where biosolids have never been applied. This is for better understanding of variation of bioaerosols 1.8 km away from the class B biosolids-applied field. Table 6 shows the changes in air temperature and wind velocity for the same dates on Riker's field as are reported in Table 5. No clear picture emerges, except possibly that wind speeds increased as winter began its onset, and temperatures dropped. The number of bacteria seems to increase with higher wind speeds, as might be expected for bioaerosols.

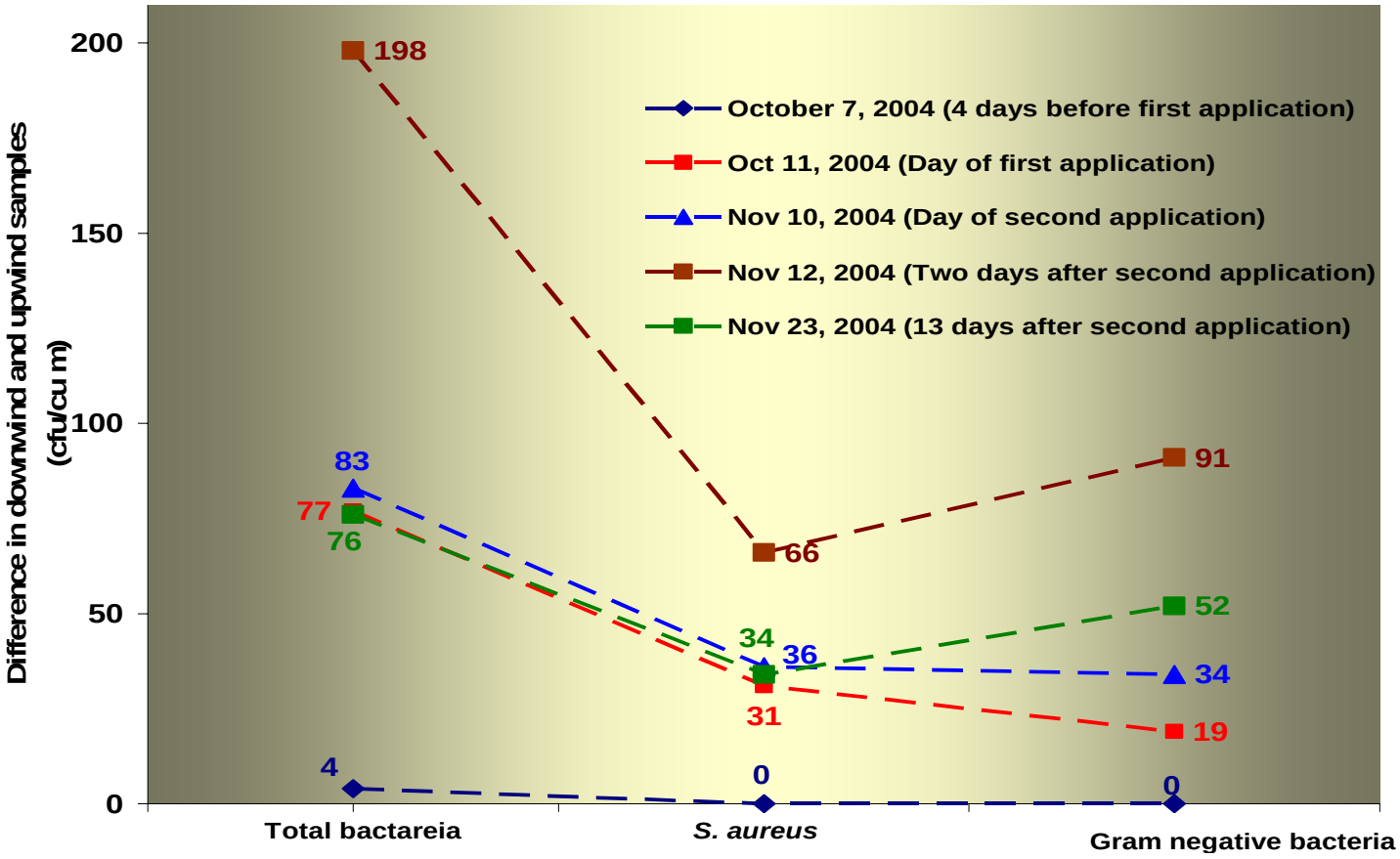

*Figure 11.*Temporal variation of bioaerosols collected at George Riker's field, Bowling Green, Ohio. All of these samples were collected by Andersen's sampler, except those on October 07, 2004 and November 23, 2004 were collected by Microflow sampler. Microflow data were normalized to the equivalent of Andersen's data using equation 1 (Page 24). Plate corrections (Equation 2, page 27) were made for the data collected by Andersen's sampler using plastic petri dishes (on November 10 and 12, 2004)

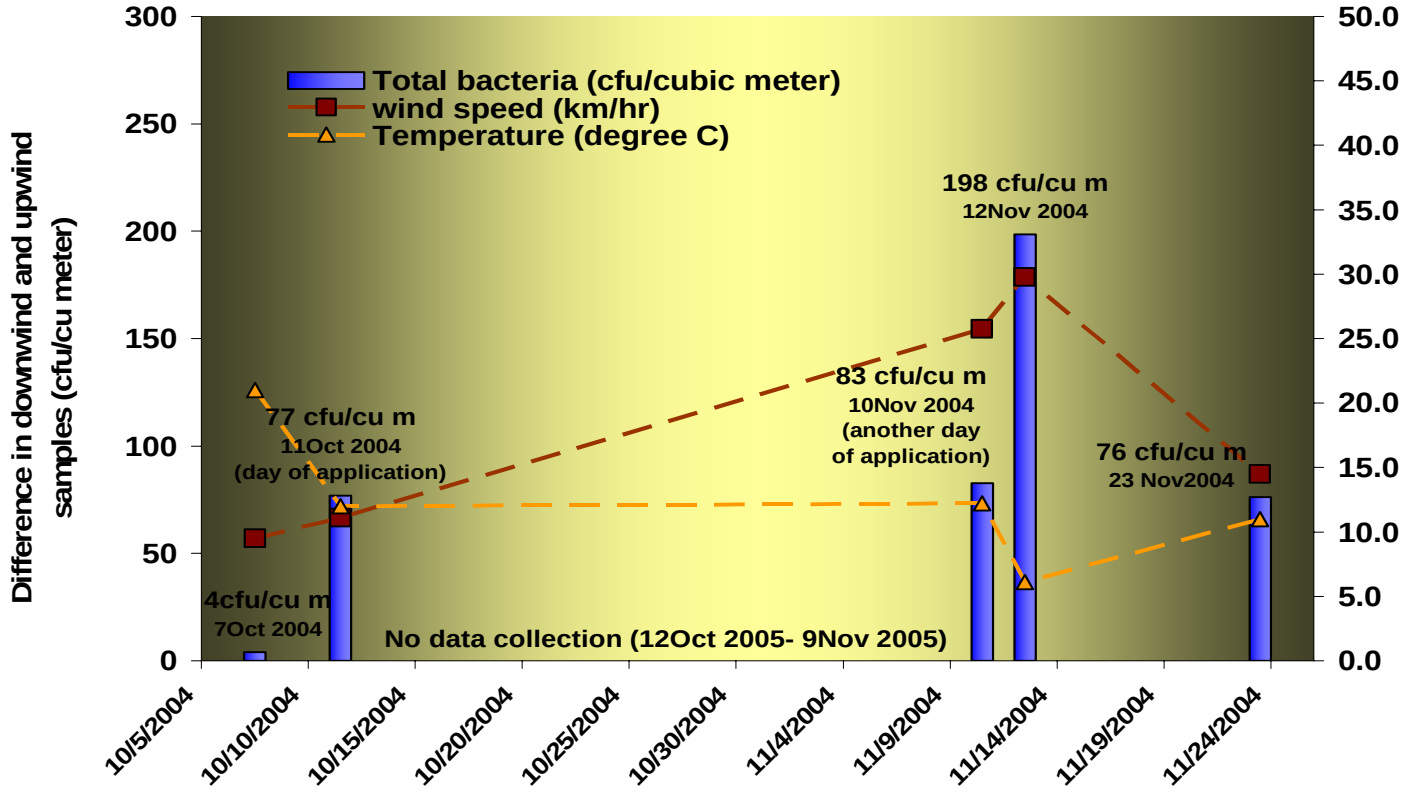

Figure 12. Difference in downwind and upwind samples of total bacteria collected at George Riker's field, Bowling Green, Ohio. October 11, 2004 and November 10, 2004 were the days of biosolids application. November 12, 2004 was 2 days after biosolids-application, and November 23, 2004 was 13 days after the application. All of these samples were collected by Andersen's sampler, except those on October 07, 2004 and November 23, 2004 were collected by Microflow sampler. Microflow data were normalized to the equivalent of Andersen's data using equation 1 (Page 24). Plate corrections (Equation 2, page 27) were made for the data collected by Andersen's sampler using plastic petri dishes (on November 10 and 12, 2004)

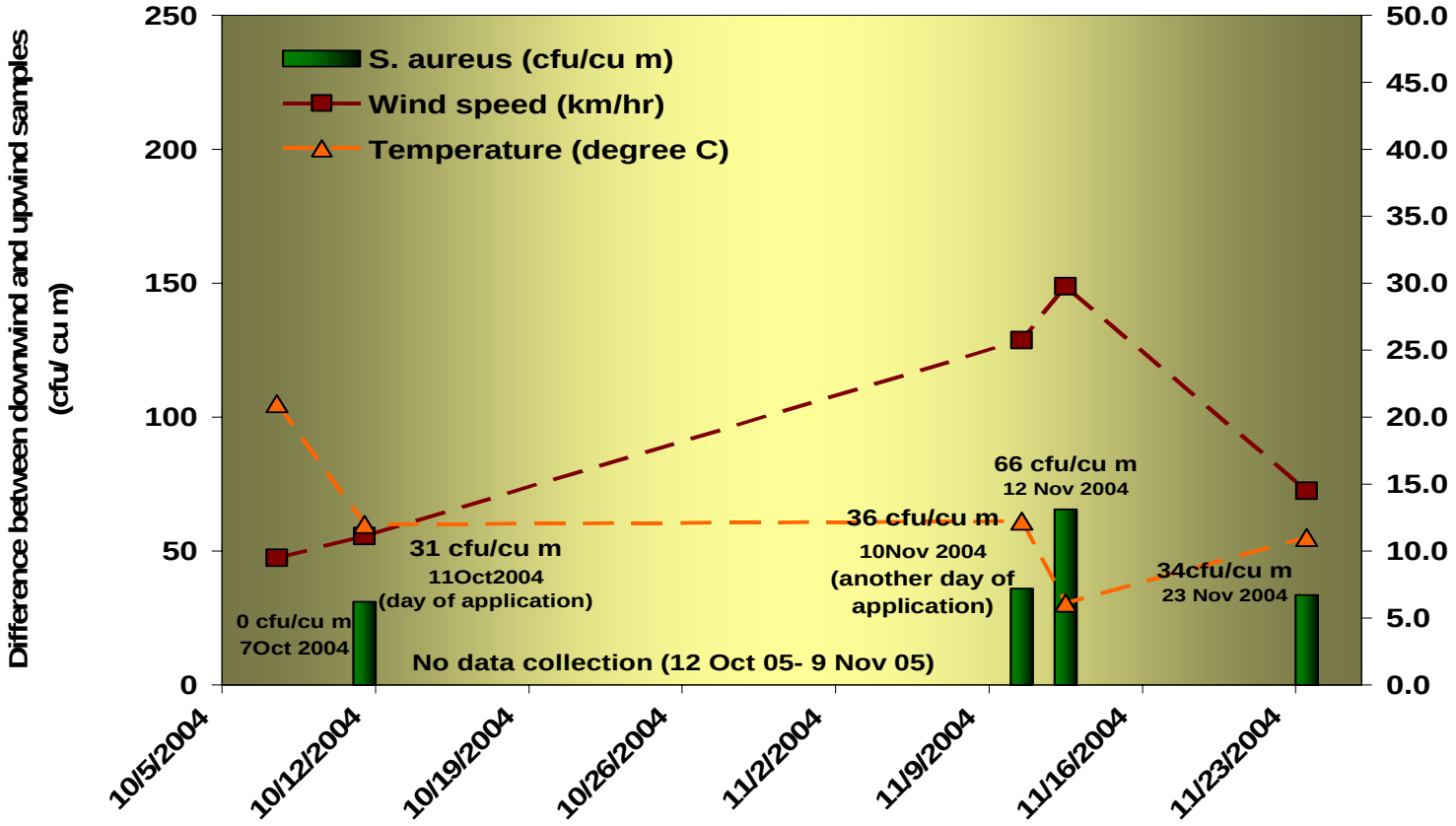

Figure 13. Difference in downwind and upwind samples of *Staphylococcus aureus* collected at George Riker's field Bowling Green, Ohio. October 11, 2004 and November 10, 2004 were the days of biosolids application. November 12, 2004 was 2 days after biosolids-application, and November 23, 2004 was 13 days after application. All of the samples were collected by Andersen's sampler, except those on October 07, 2004 and November 23, 2004 were collected by Microflow sampler. Microflow data were normalized to the equivalent of Andersen's data using Equation 1 (Page 24). Plate corrections (Equation 2, page 27) were used for the data collected by Andersen's sampler using plastic petri dishes (on November 10 and 12, 2004)

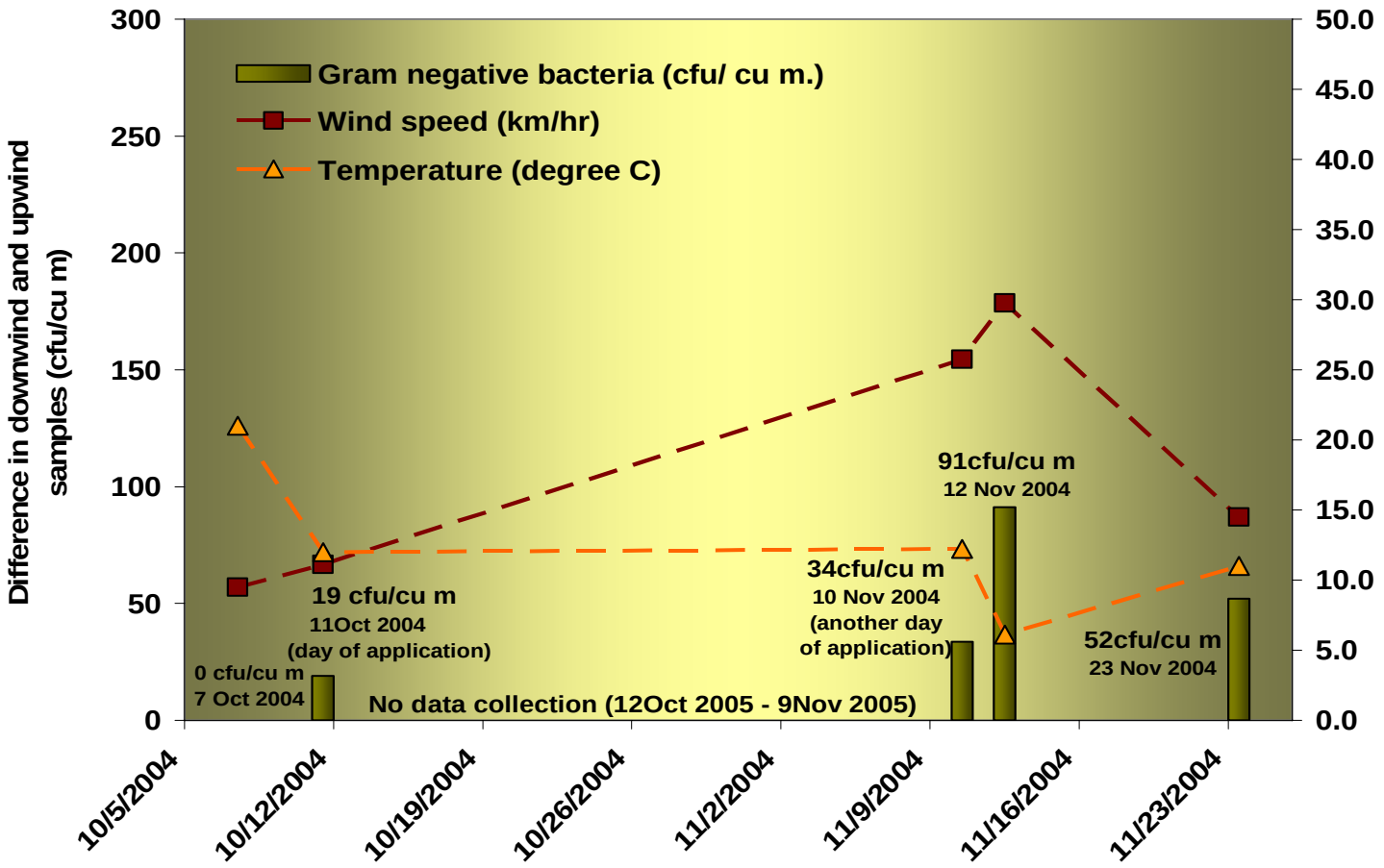

Figure14. Difference in downwind and upwind samples of gram negative bacteria from downwind and upwind samples collected at George Ryker's field Bowling Green, Ohio. October 11, 2004 and November 10, 2004 were the days of biosolids application. November 12, 2004 was 2 days after biosolids-application, and November 23, 2004 was 13 days after application. All of the samples were collected by Andersen's sampler except those on October 07, 2004 and November 23, 2004 were collected by Microflow sampler. Microflow data were normalized to the equivalent of Andersen's data using Equation1 (Page 24). Plate corrections (Equation 2, page 27) were used for the data collected by Andersen's sampler using plastic petri dishes (on November 10 and 12, 2004).

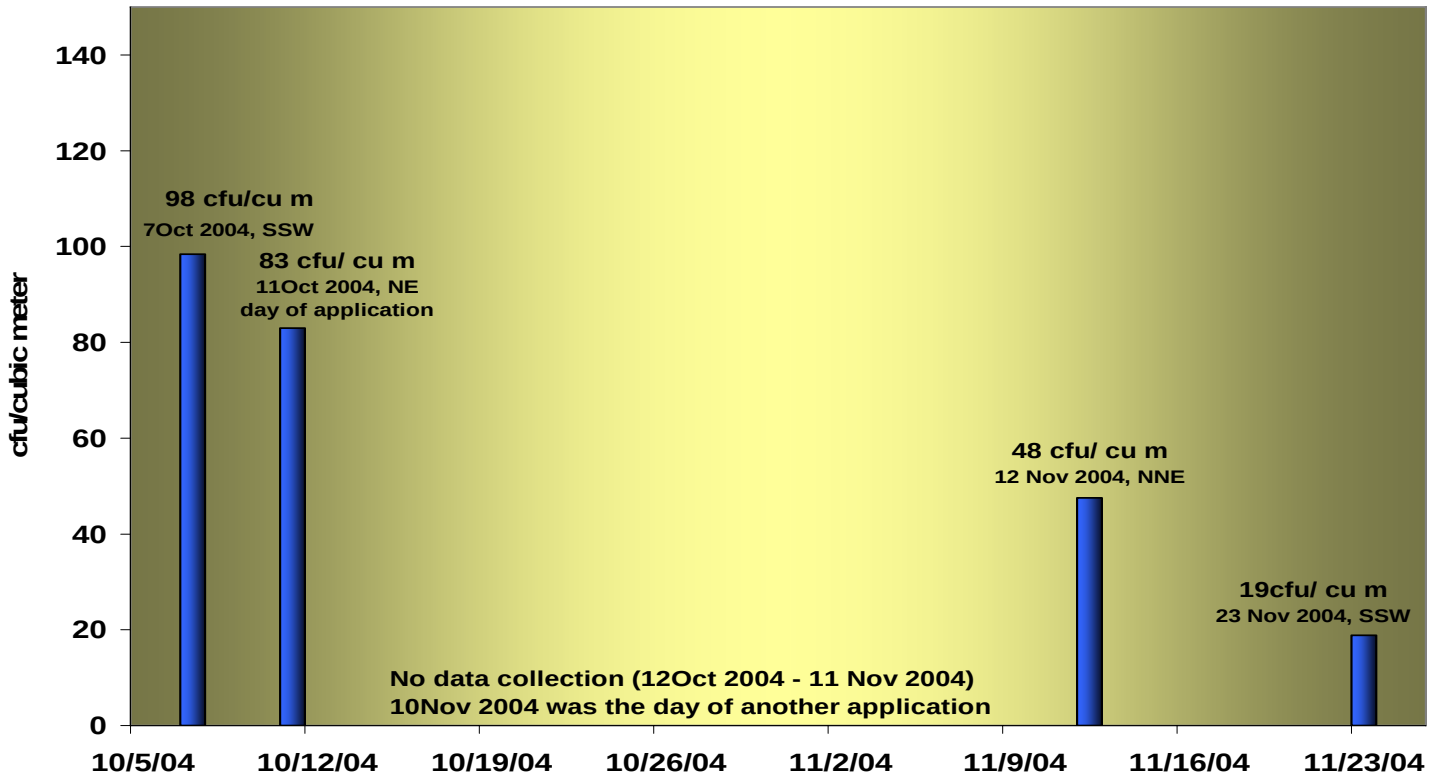

Figure 15. Temporal variation in total bacteria collected at the football stadium, Bowling Green, Ohio. The dates of collection, and the directions from which the wind was blowing, have been shown. October 11, 2004 was the day of biosolids application at Riker's field, November 12, 2004 was 2 days after the application, and November 23, 2004 was 13 days after application. However, no data was collected on November 10, 2004, which was another day of application at Riker's field. All of the samples were collected by Andersen's sampler except that those on October 07, 2004 and November 23, 2004 were collected by Microflow sampler. Microflow data were normalized to the equivalent of Andersen's data using Equation 1 (Page 24).

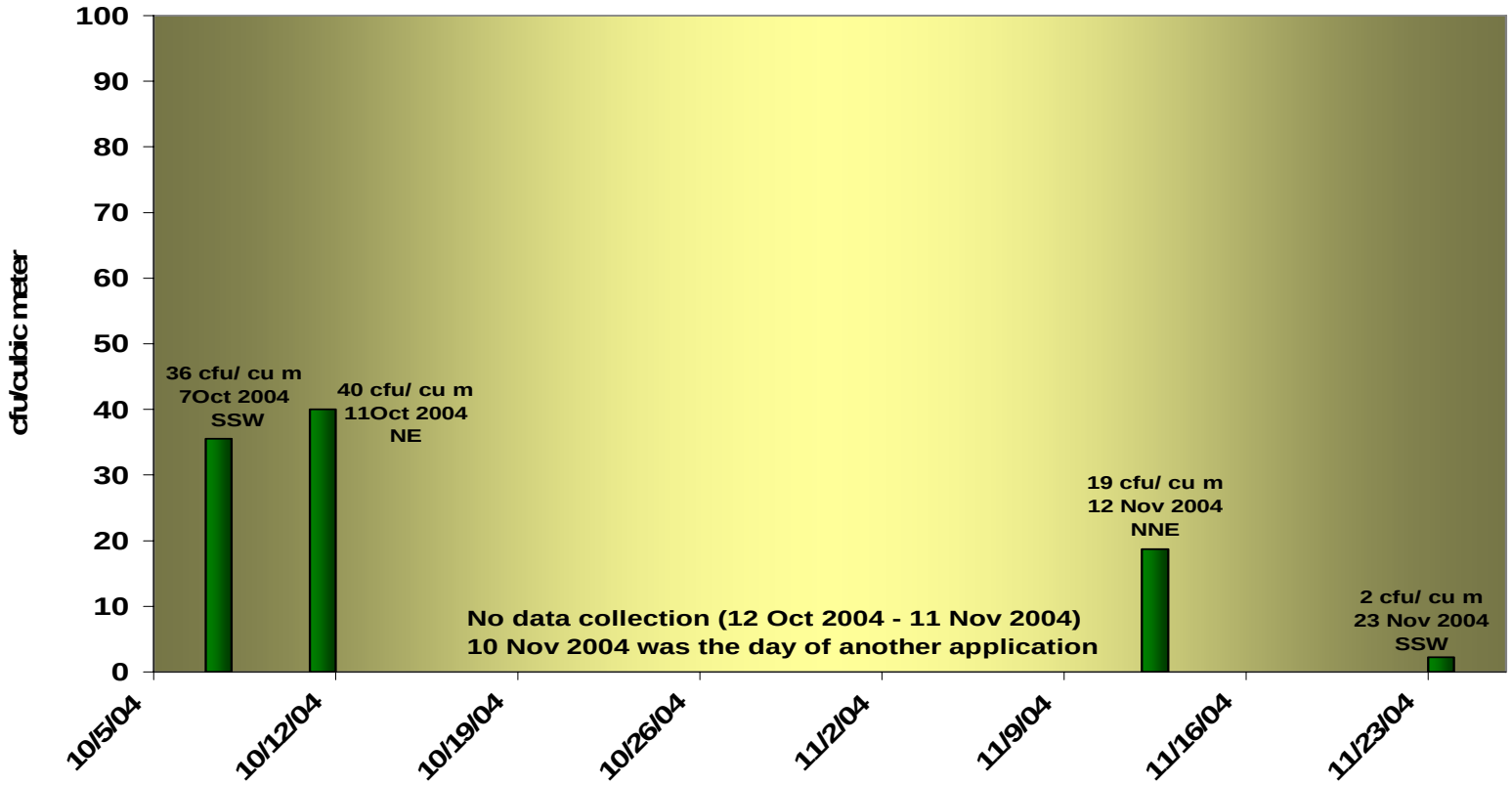

*Figure 16.*Temporal variation in *S. aureus* collected at the football stadium, Bowling Green, Ohio. The dates of collection and the directions from which the wind was blowing have been shown. October 11, 2004 was the day of biosolids application, November 12, 2004 was 2 days after application, and November 23, 2004 was 13 days after application. However no data was collected on November 10, 2004, which was the day of another application at Riker's field. All of the samples were collected by Andersen's sampler, except that those on October 07, 2004 and November 23, 2004 were collected by Microflow sampler. Microflow data were normalized to the equivalent of Andersen's data using Equation 1 (Page 24).

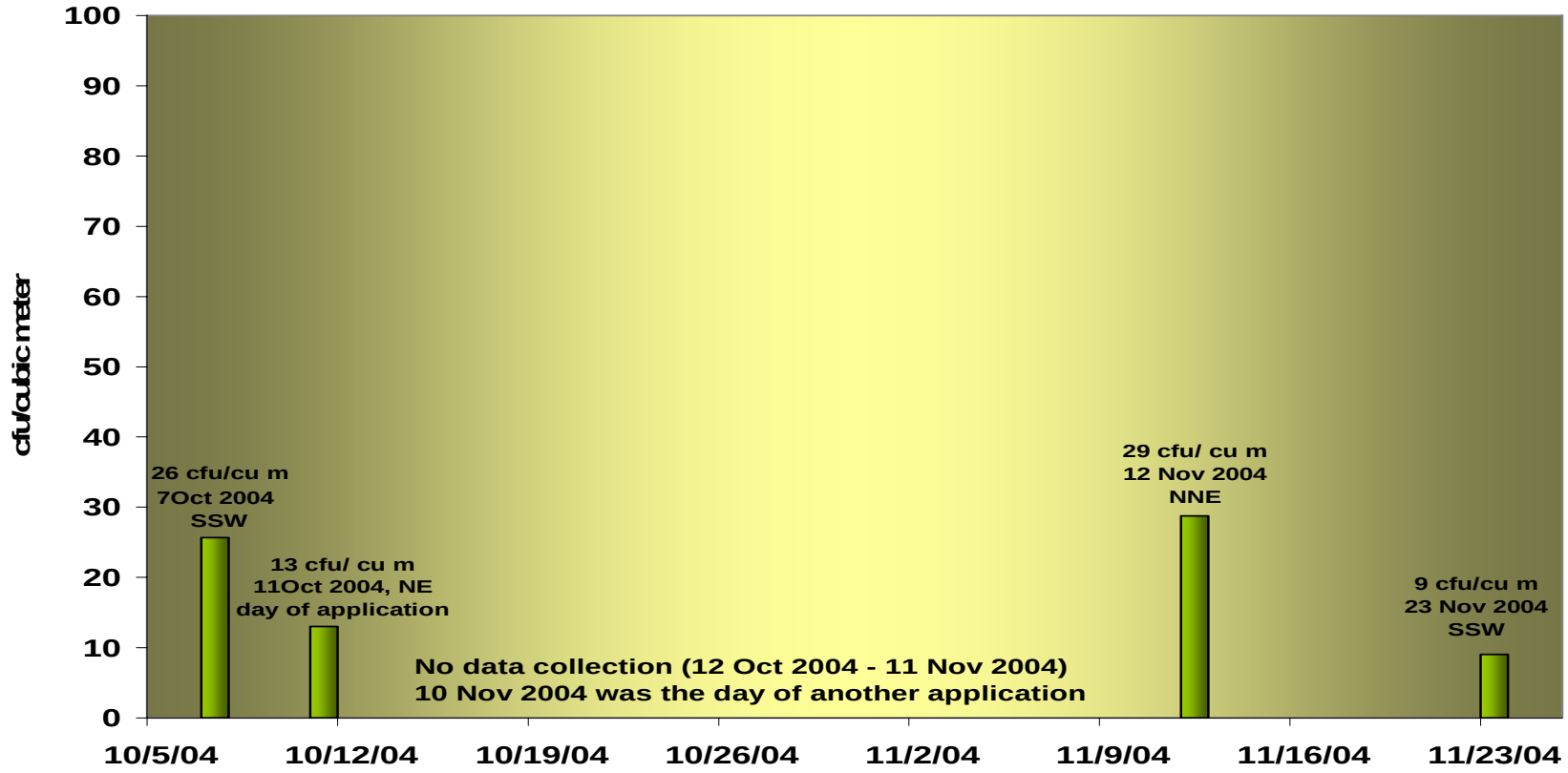

Figure 17. Temporal variation in Gram negative bacteria collected at the football stadium, Bowling Green, Ohio. The dates of collection, and the directions from which the wind was blowing, have been shown. October 11, 2004 was the day of biosolids-application, November 12, 2004 was 2 days after application, and November 23, 2004 was 13 days after application. However, no data was collected on November 10, 2004, which was another day of biosolids application. All of the samples were collected by Andersen's Sampler, except that those on October 07, 2004 and November 23, 2004 were collected by Microflow sampler. Microflow data were normalized to the equivalent of Andersen's data using Equation 1 (Page 24).

Table 5. *Difference in Downwind and Upwind Bioaerosols Samples for 5 Different Dates*

Date	Total bacteria (cfu/cu m)	S. aureus (cfu/cu m)	Gram negative Bacteria (cfu/cu m)
10/07/2004	4	0	0
10/11/2004	77	31	19
11/10/2004	83	36	34
11/12/2004	198	66	91
11/23/2004	76	34	52

Table 6.*Record of Temperature (˚C) and Wind Speed (km/hr) for 5 different dates.*

Date	Temperature (˚C)	Wind speed (km/hr)
10/07/2004	21	9.5
10/11/2004	12	11.1
11/10/2004	12	25.7
11/12/2004	6	29.8
11/23/2004	11	14.5

It should be noted that number of cfus of total bacteria, and the total gram-negative bacteria from the football stadium were comparatively higher (Figures 15 and 17) 4 days prior to the application (10/07/2004) of biosolids in Riker's field. Total number of bacteria and gram-negative bacteria were calculated as 98 cfu/cubic meter and 26 cfu/cubic meter, respectively, on 10/07/2004, whereas, they were less, 83 cfu/cubic meter and 13cfu/cubic meter, respectively, on the day of class B biosolids application (10/11/04). The wind was blowing from the football stadium (from SSW) toward Riker's Field and the WWTP at that particular time (10/07/2004). About 25000 people were enjoying a football game at the football stadium on that day, which might have accounted for the elevated levels of bioaerosols, because the human nose and skin are abundant sources of bacteria. Another possibility may be any illegal application of biosolids at a nearby field, which was not officially reported. However, it is important to note that the actual number of bacterial colonies (the difference in numbers of colonies collected from down wind

and up wind) at the biosolids-applied field (George Riker's field) was less than the day of application (Figures 11, 12, 13, and 14).

Due to their lighter weight, finer particles can be transported for long distances. As the Andersen's sampler acts like an aerodynamic filter, the higher order plates collect the finer particles. On the basis of this assumption, an investigation was carried out to explore whether the finer particles could be carried up to a distance of 1.8 km (1.1 miles, the distance of the football stadium from Riker's field). Tables 7 and 8 show the percentage of cfu/cubic meter collected in first and second order plates of Andersen's Sampler added together with respect to the cfu/cubic meter collected by all plates added together. The percentage of third and fourth order plates together and fifth and sixth plates together are also shown in the tables. These data were collected on 10/11/04 and 11/12/04, when the wind was blowing from a NE to NNE direction; therefore, the football stadium was approximately in the downwind direction from Riker's field. The particle density collected at the upwind, downwind, and the football stadium show a higher percentage in higher order plates (in 95% of the samples).

Only staphylococcus was higher in number as collected from the football stadium (Figure 16) on the day of class B biosolids application (10/11/04) at Riker's field. The wind was blowing from the NE on that particular day, and the Football stadium was directly downwind from Riker's field. However, contamination from the bioaerosols generated by the Bowling Green WWTP, which is located few meters NE of the football stadium, should also be taken into consideration. Except for staphylococcus, no other bacteria at the football stadium were higher on the day of application. Also, no data were collected from the Bowling Green WWTP, which could have been an important source of bioaerosols at the football stadium. The previous

discussion suggests that further investigation is needed in order to conclude that the higher percentage of finer particles at the football stadium was actually originated in Riker's field.

Table 7. *Percentage of cfu/cubic meter Collected at Various Ordered Plates on 10/11/2004 (Date of Biosolids Application) at Riker's Field and Football Stadium, Bowling Green, Ohio.*

Total Bacteria

Location	Football Stadium				Riker's Field Upwind				Riker's Field Downwind			
Plate no.	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total
cfu/cubic meter	2	46	35	83	16	18	5	39	0	46	70	116
Percentage	2	55	42	100	41	46	13	100	0	40	60	100

S. aureus

Location	Football Stadium				Upwind				Downwind			
Plate no.	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total
cfu/cubic meter	2	23	15	40	15	17	6	38	0	21	48	69
Percentage	5	58	38	100	39	45	16	100	0	30	70	100

Gram Negative

Location	Football Stadium				Upwind				Downwind			
Plate no.	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total
cfu/cubic meter	0	4	9	13	0	2	0	2	0	5	16	21
Percentage	0	31	69	100	0	100	0	100	0	24	76	100

Table 8. *Percentage of CFU/Cubic Meter Collected at Various Ordered Plates, Collected on 11/12/2004 (2 Days After Biosolids Application) at Riker's Field and Football Stadium, Bowling Green, Ohio.*

Total Bacteria

Location	Football Stadium				Riker's Field Upwind				Riker's Field Downwind			
Plate no.	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total
cfu/cubic meter	8	11	19	38	35	40	37	112	72	65	102	239
Percentage	21	29	50	100	31	36	33	100	30	27	43	100

S. aureus

Location	Football Stadium				Riker's Field Upwind				Riker's Field Downwind			
Plate no.	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total
cfu/cubic meter	4	5	5	14	4	12	21	37	28	21	30	79
Percentage	29	36	36	100	11	32	57	100	35	27	38	100

Gram negative bacteria

Location	Football Stadium				Riker's Field Upwind				Riker's Field Downwind			
Plate no.	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total	1+2	3+4	5+6	Total
cfu/cubic meter	4	5	14	23	23	19	14	56	40	32	58	130
Percentage	17	22	61	100	41	34	25	100	31	25	45	100

Selection of biological instruments is also an important factor for research with bioaerosols. As discussed previously, both Andersen's six-stage sampler and the Microflow one-stage sampler are impactors. Therefore, attenuation of the number of colonies with increased sampling time is always a possibility. To avoid this predicament, Rusin et al. (2005) used an SKC air sampler, which is an impinger that collects the bacteria in a swirling liquid medium, which reduces the particle bounce and re-aerosolization.

Rusin et al. (2003) confirmed an absence of *S. aureus* from aerosols generated from class B biosolids. In contrast, this thesis research found an average of 55 cfu/cubic meter in the downwind in comparison to 30 cfu/cubic meter from the upwind direction on the day of application. The samples collected during post application days (2 days and 13 days after class B biosolids application) show an average of 66 cfu/cubic meter in downwind and only 12 cfu/cubic meters from the upwind direction. The results observed by Rusin et al. (2003) are weakened because they have experimentally shown that they used the most probable number method (MPN) with only 8.7% efficiency for recovering the *S. aureus* from the samples.

It should also be noted that the MPN method provides an estimation of the probable number of aerosolized bacteria cells; whereas, Andersen's sampler collects the dust particles that contain one or more bacteria cells on its surface. Andersen (1958) has shown that if the sampled particles are very fine (and if it is assumed that one single particle contains one single cell), an impinger will have approximately 50% less efficiency for bioaerosols collection than an Andersen's sampler.

Table 9 shows the average surface area of the particles collected in the plates of various order. The pore size range of the Andersen's sampler varies from a maximum of $\geq 7.0\mu$ in the first plate to a minimum of $1.1\mu - 0.65\mu$ (Figure 5) in the sixth plate (at the bottom). Therefore,

the particle surface area collected by this sampler varies from 153.9 μ^2 at the first and second plates to as small as 3.8 μ^2 at the sixth plate. Because the average surface area of a bacterium is 3.1μ^2 (average diameter of a bacterium is 1μ), it is theoretically possible that the Andersen's sampler in plates 1 and 2 can collect bacteria cells at least 153.9/3.1 = 49.6 times higher than the number of particles. Whereas, the bacteria cells collected per particle in the third and fourth plates can be as much as 34.2/3.1 = 11.0 times higher than the number of particles that have bacterium on it (measured as one colony forming unit). However, the particles collected in the fifth and sixth plates may contain approximately the same number of cells (3.8/3.1 = 1.2) as the cfu. This implies that the colony forming units measured from the impactors are greatly underestimating the actual number of bacteria. As a result the number of cfu's should be multiplied by a factor to estimate the number of cells before the results of an impactor is compared with the MPN estimate from impinger data collection results.

Table 9. *Approximate Surface Area of the Particles (Assumed as Spherical) Collected in Different Plates of Andersen's Sampler.*

Plate Number	Average Diameter (μ)	Surface area (μ^2)
1+2	7.0	153.9
3+4	3.3	34.2
5+6	1.1	3.8

Note. The intermediary-diameter of the two plates used as the average diameter. The surface area of a spherical particle is equal to πD^2, where D is the diameter.

Rusin et al (2003) collected the samples from the locations with a relative humidity ranging from 4% to 50% and a wind speed ranging from 1.8 km/hr to 12.9 km/hr. By contrast, in Bowling Green, Ohio, the average afternoon humidity ranged from 57% to 66%, and wind speed varied from 9.5 km/hr to 29.8 km/hr. The researcher thinks that these environmental factors could have increased the amount of *S. aureus* in bioaerosols collected in Bowling Green relative to those of Rusin et al. (2003).

An investigation conducted by Tanner et al. (2005) reported no coliforms (gram-negative bacillus) on the day of application of class B biosolids. However, they also reported that at low humidity level (16% in Arizona), *E. coli* (gram-negative bacteria) quickly lose their viability after aerosolization. In this thesis investigation, the total number of gram-negative bacteria averaged 26 cfu/cubic meter from downwind and 3 cfu/cubic meter from upwind on the day of application. An average of 123 cfu/cubic meter and 39 cfu/cubic meter were detected from downwind and upwind samples, respectively, on post application dates, showing a growth in gram-negative bacteria for at least 2 days after biosolids-application.

CONCLUSIONS

S. aureus and gram-negative bacteria (including *E. coli)*, which are potentially pathogenic, were commonly found in the aerosols generated from class B biosolids-applied fields and the class B waste water treatment plant at Bowling Green, Ohio. Higher bacterial counts were always found in the downwind directions compared to the upwind directions. The differences between these two counts (upwind and downwind) have been considered to be the bioaerosols generated from the applied fields. These values were used to study temporal variation with respect to before and after the biosolids application in the farm field. However, we have only circumstantial evidence that they were generated from the biosolids-applied field itself, perhaps including contamination from outside sources. A genetic correlation between the bacteria collected from the soil samples and the bioaerosols, could explicitly prove if the bioaerosols were originated from the biosolids applied field.

Maximum increase in bacterial count was documented after 2 days of biosolids application. On the 2 days of class B biosolids application an average of 71 cfu/cubic meter of total bacteria, 30 cfu/cubic meter of *S. aureus*, and 26 cfu/cubic meter of gram-negative bacteria were found. Two days after class B biosolids application, the numbers increased up to 159 cfu/cubic meter of total bacteria, 53 cfu/cubic meter of *S. aureus*, and 91 cfu/cubic meter of gram negative bacteria, respectively.

Higher number of colonies was always documented from the data collected in Microflow sampler than Andersen's sampler. A linear relationship has been established between the data collected by these two samplers, and it was used to normalize the entire data equivalent to Andersen's data. All these data have been used to study the temporal variation of bioaerosols generated from biosolids-applied fields. The R^2 value for the best fit straight line is

approximately 0.7, which illustrates a strong linear correlation between the total numbers of colonies collected by the two samplers. The root mean square value is approximately 30 cfu/cubic meter.

Although there was an increase in bacterial count 2 days after the class B biosolids application, it decreased in terms of total bacterial content (133 cfu/cubic meter) and gram-negative bacteria (52 cfu/cubic meter), 13 days after the application. However, *S. aureus* showed a continuous increase of up to 64 cfu/cubic meter after 13 days of application. A further study, incorporating sampling on later dates of a post-application period is needed to determine the long-term fate of *S. aureus* produced by class B biosolids application in the farm fields.

Rusin et al. (2003) confirmed an absence of *S. aureus* from aerosols generated from class B biosolids, whereas this thesis research found an average of 55 cfu/cubic meter downwind in comparison to 30 cfu/cubic meter from the upwind direction on the day of application. The samples collected during post-application days (2 days and 13 days after class B biosolids application) show an average of 66 cfu/cubic meter in downwind and only 12 cfu/cubic meters from the upwind direction. Rusin et al. have used the MPN method for *S. aureus* count with an efficiency of only 8.7%. Andersen (1958) has shown that if the sampled particles are very fine (and if it is assumed that one single bioaerosol particle contains one single cell), an impinger will have approximately 50% less efficiency than Andersen's sampler, with respect to bioaerosol collection. These findings may partially explain the cause of absence of *S. Aureus* collected with an impinger by Rusin et al.

Andersen's sampler collects finer particles at higher order filter plates (such as in third, fourth, fifth, and sixth plates). Samples collected from the football stadium (1.8 km apart from Riker's field), Bowling Green, Ohio contain high percentages of bacteria in terms of total

bacteria and gram-negative bacteria (more or less 50%), in higher order plates. For both days (on the day of application and 2 days after class B biosolids application), the football stadium was directly downwind from Riker's field (NE-NNE wind); therefore, these results imply that the finer particles can be transported for an extended distance. However, no robust conclusion can be drawn from this data, because the temporal variation study at the football field does not completely match with that from the class B biosolids-applied field. An intensive study with more data can give a possible idea of the transportation of bioaerosols from the biosolids-applied farm field and its impact on the residents kilometers away from the class B biosolids-applied field.

This research indicates that not only the workers applying biosolids in the field, but also local residents have the possibility of being affected by the bioaerosols generated from class B biosolids, and the rate of vulnerability increases during the post-application period. However, there is no standard absolute concentration, suggested by the U.S. EPA, above which *S. aureus* and gram-negative bacteria can be harmful to humans. If class A biosolids generate bioaerosols that are greatly lower in *S. aureus* and other human pathogens, as is expected to occur, considerations should be given to substituting class A biosolids for class B biosolids in application for all farm fields in Ohio.

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APPENDIX – I

SAMPLES COLLECTED ON DIFFERENT DATES AT VARIOUS PLACES OF BOWLING GREEN, OHIO

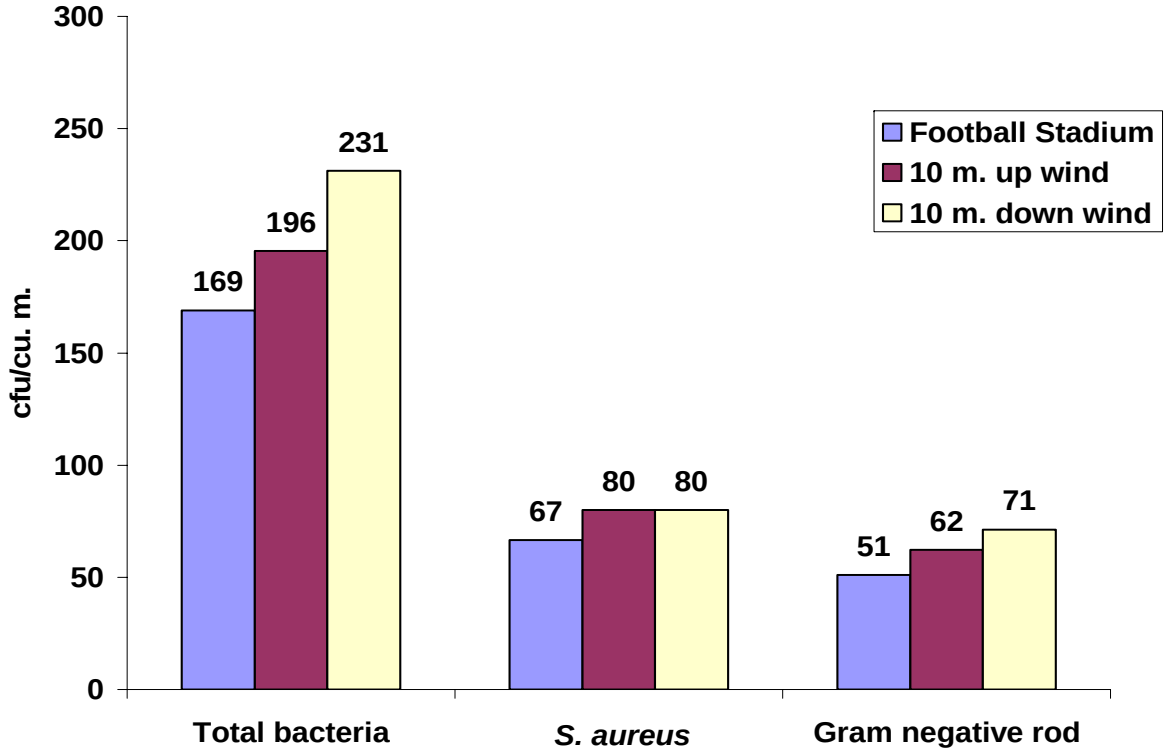

Figure AI-1.Bacteria collected in Microflow sampler (single stage sampler) on October 07, 2004 (four days before biosolids-application at Riker's field). The bacteria were collected at the Football stadium, Bowling Green Ohio, and from the upwind and downwind directions at George Riker's field, Bowling Green Ohio. The noon temperature on this date was 21.1˚C with winds from south-south-west (SSW).

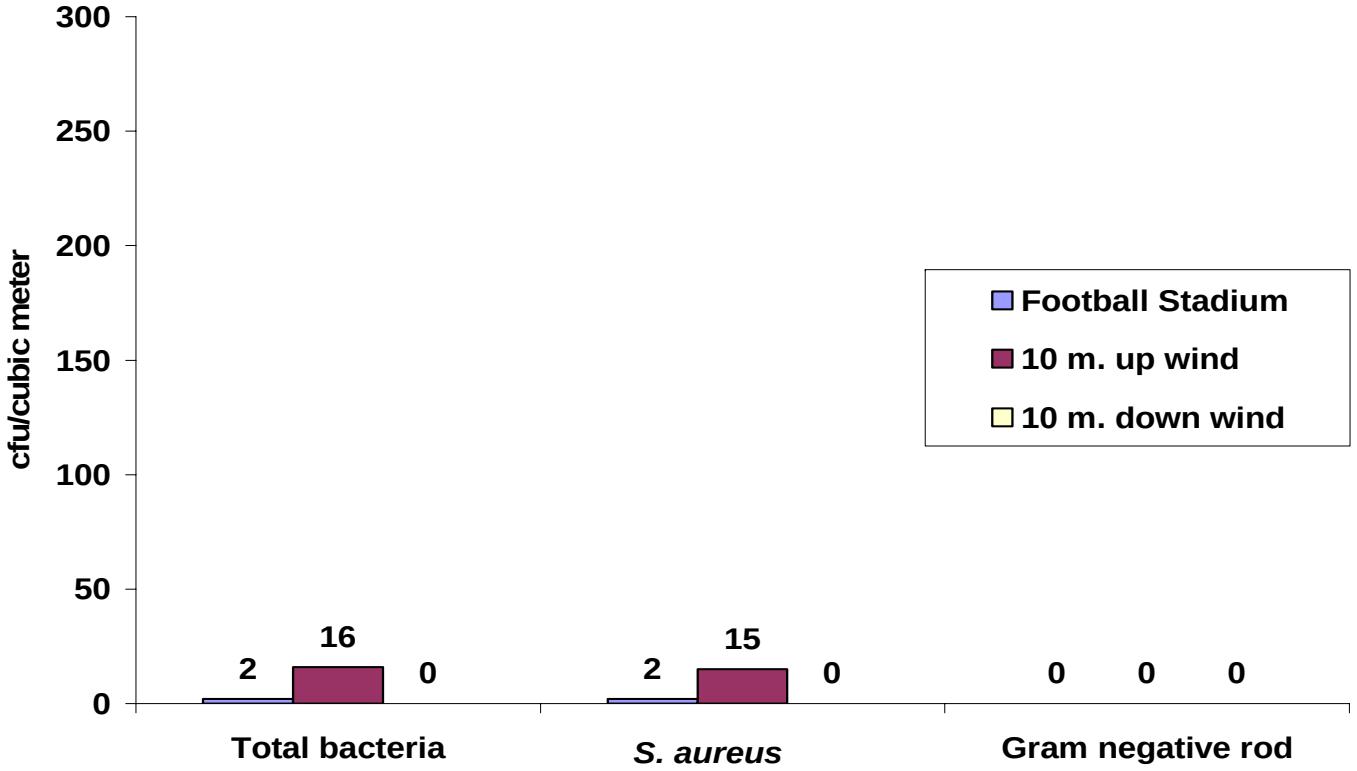

Figure AI-2.Bacteria collected in first and second plates together of Andersen's sampler on October 11, 2004, (During biosolids-application at Riker's field). It was collected at Football stadium, Bowling Green Ohio, and from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature on this date was 17.2ºC with the wind from north east (NE).

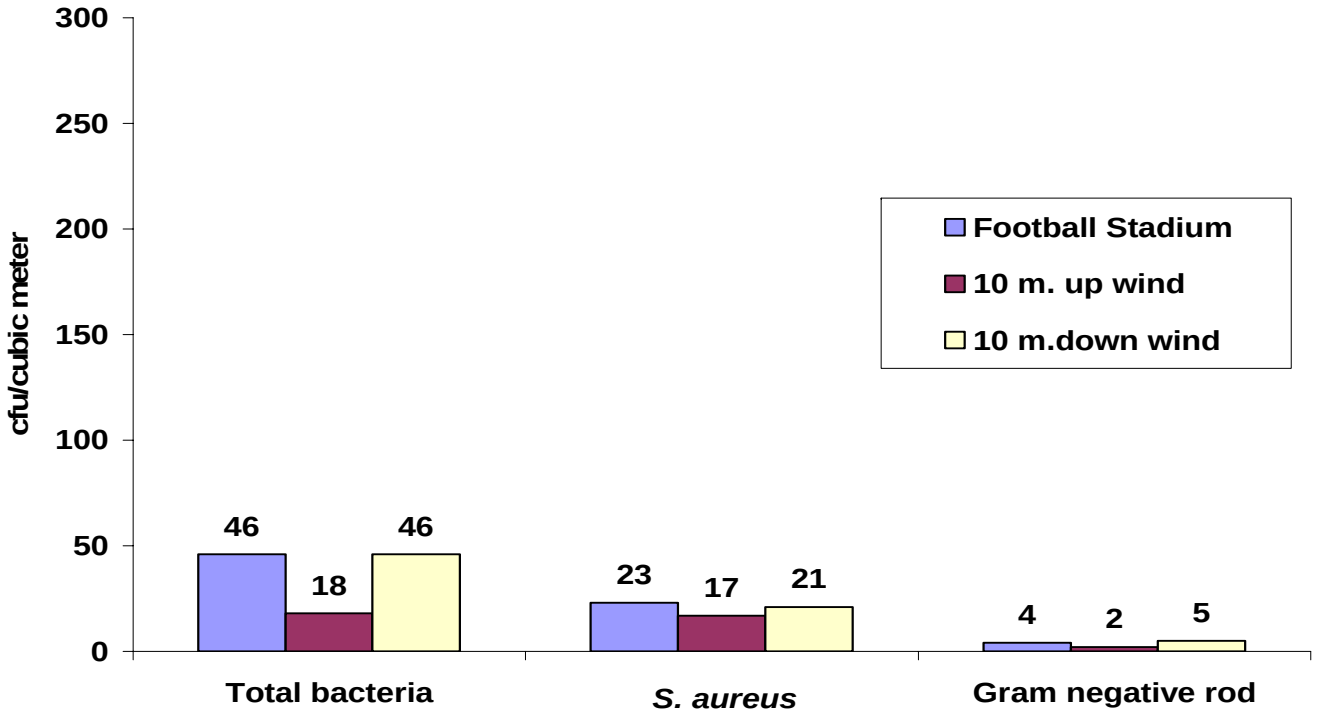

Figure AI-3.Bacteria collected in third and fourth plates together of Andersen's sampler on October 11, 2004 (during biosolids-application at Riker's field). It was collected at the Football stadium, Bowling Green Ohio, and from the upwind and downwind directions at George Riker's field Ohio. The noon temperature on this date was 17.2º C with the wind from NE.

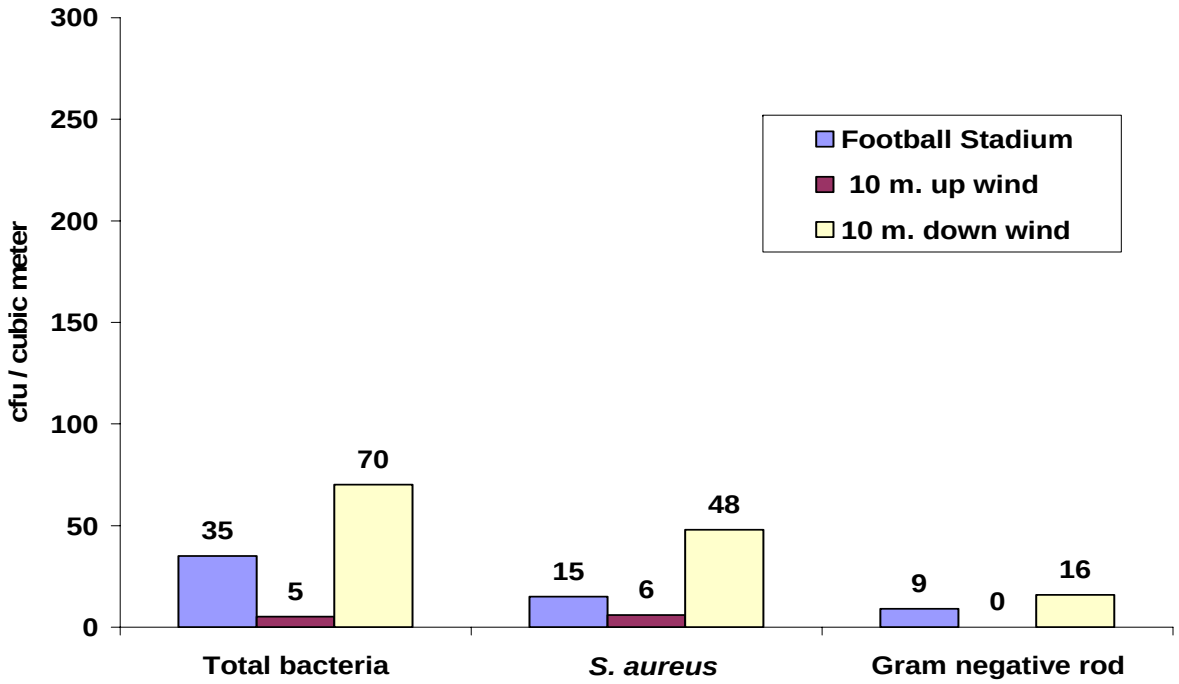

Figure AI-4.Bacteria collected in fifth and sixth plates together of Andersen's sampler on October 11, 2004, (During biosolids-application at Riker's field). It was collected at the Football stadium, Bowling Green, Ohio, and from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature on this particular date was 17.2ºC with the wind from NE.

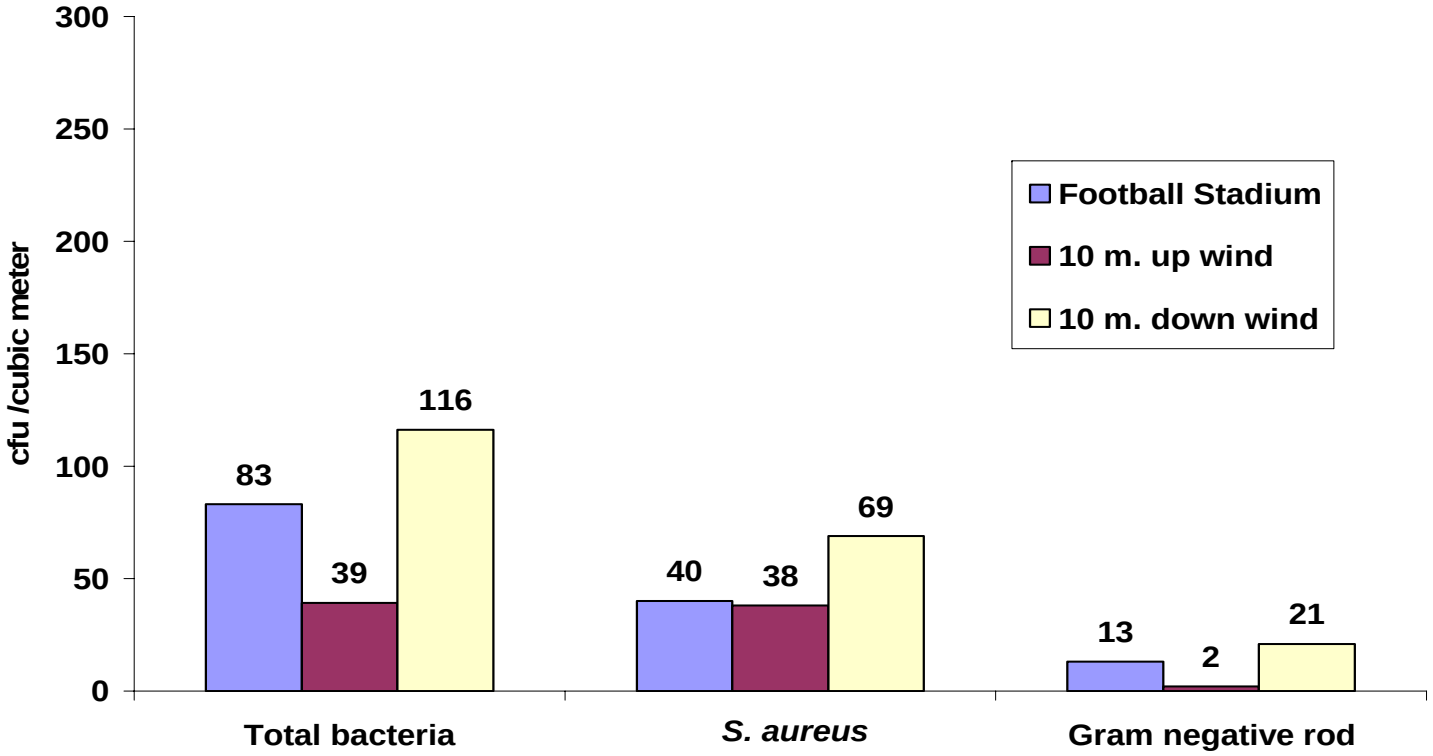

Figure AI-5.Bacteria collected in all plates together of Andersen's sampler on October 11, 2004, (During Biosolids-application at Riker's field). It was collected at the Football stadium, Bowling Green Ohio, and from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature on that particular date was 17.2º C with the wind from NE.

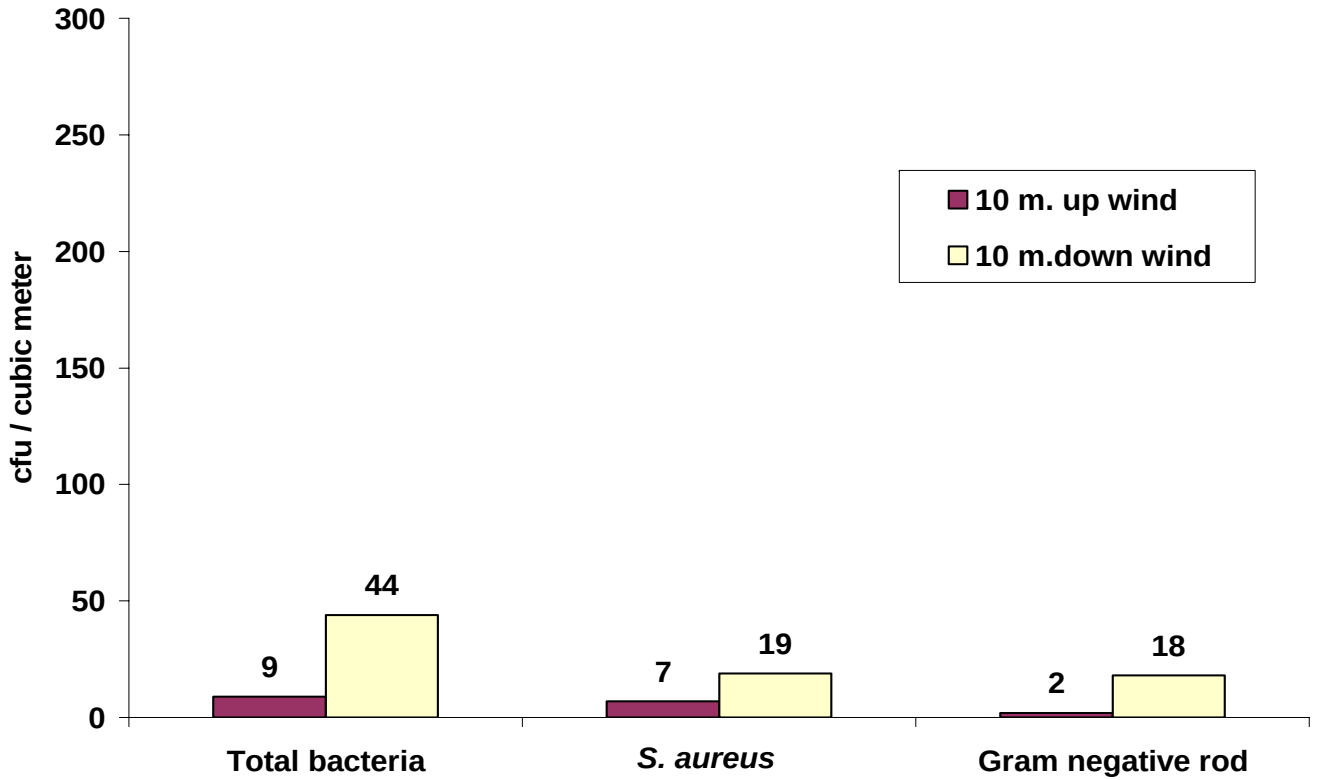

Figure AI-6.Bacteria collected in first and second plates together of Andersen's sampler on November 10, 2004 (During biosolids-application at Riker's field).The samples were collected from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature on that particular date was 12ºC with the wind from NNE- NE.

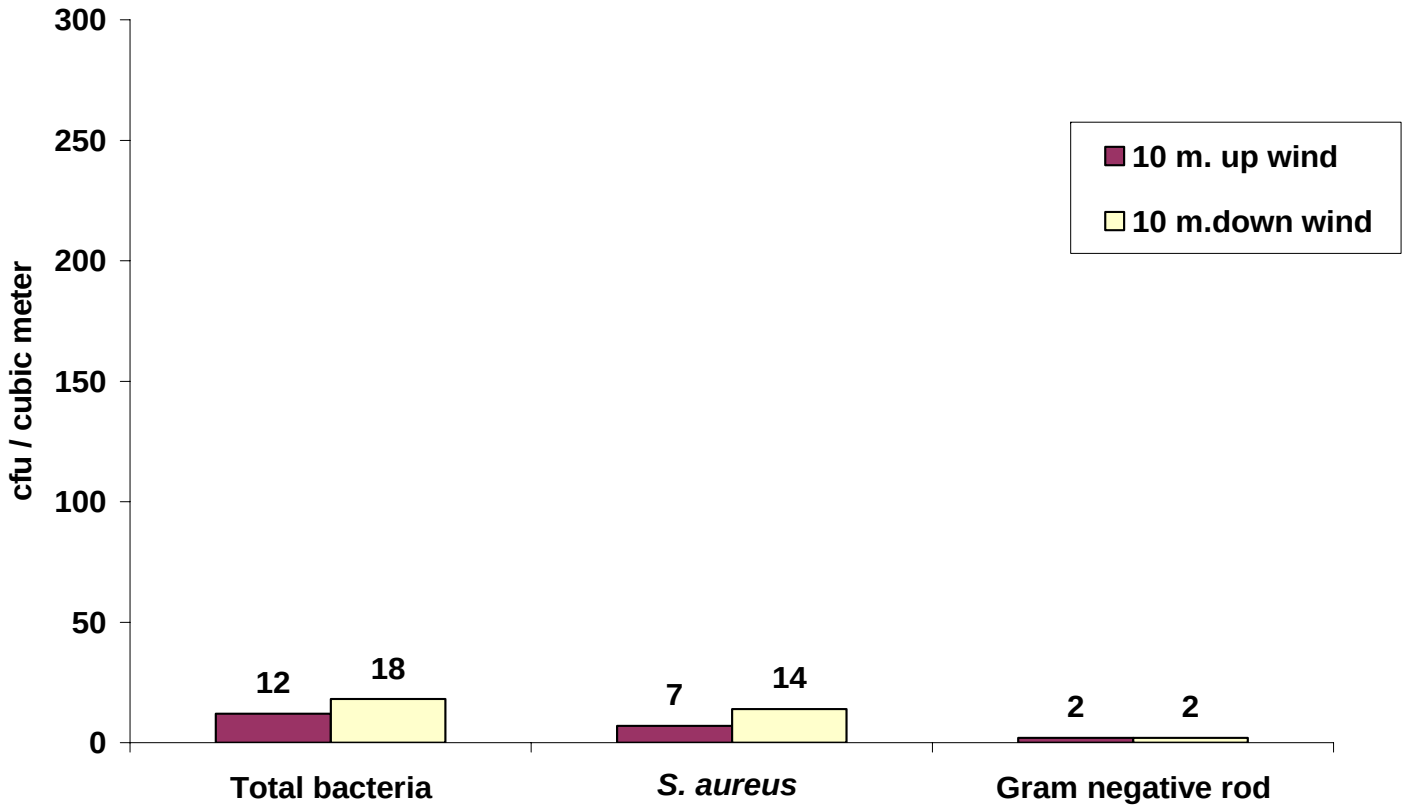

Figure AI-7.Bacteria collected in third and fourth plates together of Andersen's sampler on November 10, 2004 (During biosolids-application at Riker's field). The samples were collected from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature on that particular date was 12ºC with the wind from NNE- NE.

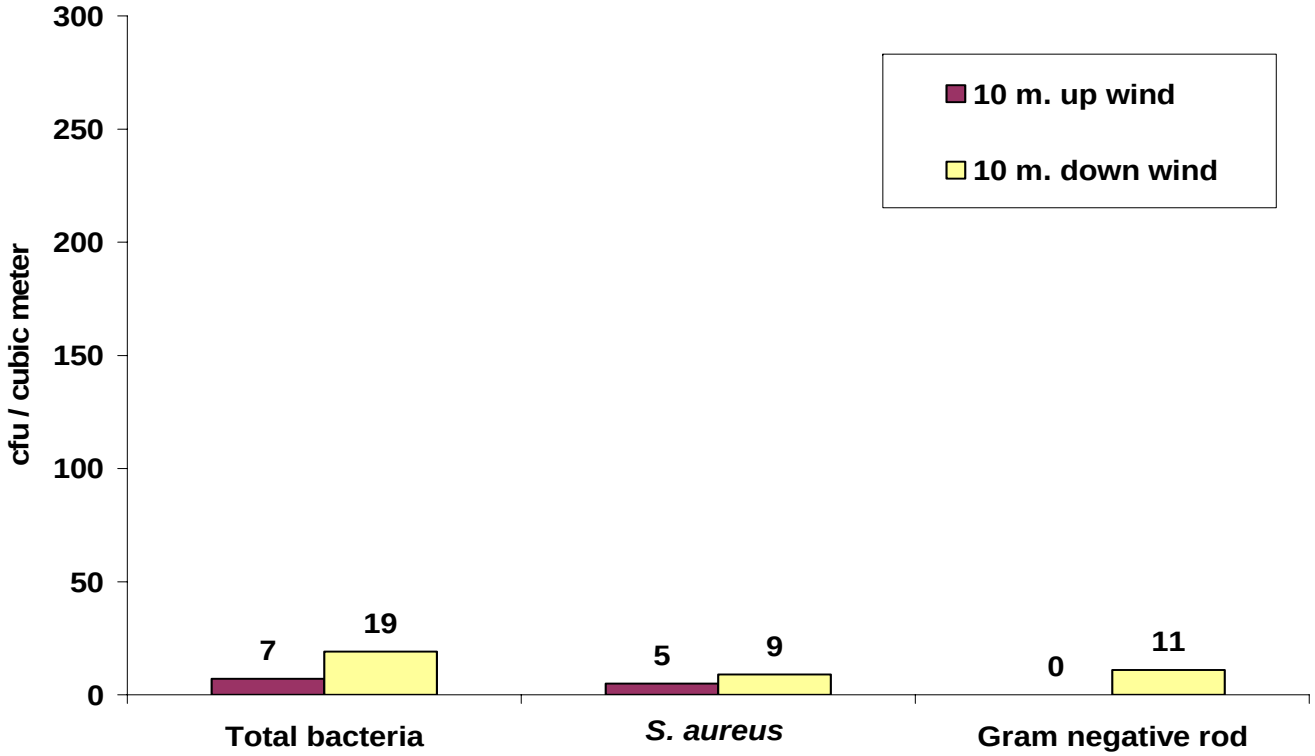

Figure AI-8.Bacteria collected in fifth and sixth plates together of Andersen's sampler on November 10, 2004 (During biosolids-application at Riker's field). The samples were collected from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature for this date was 12ºC with the wind from NNE- NE.

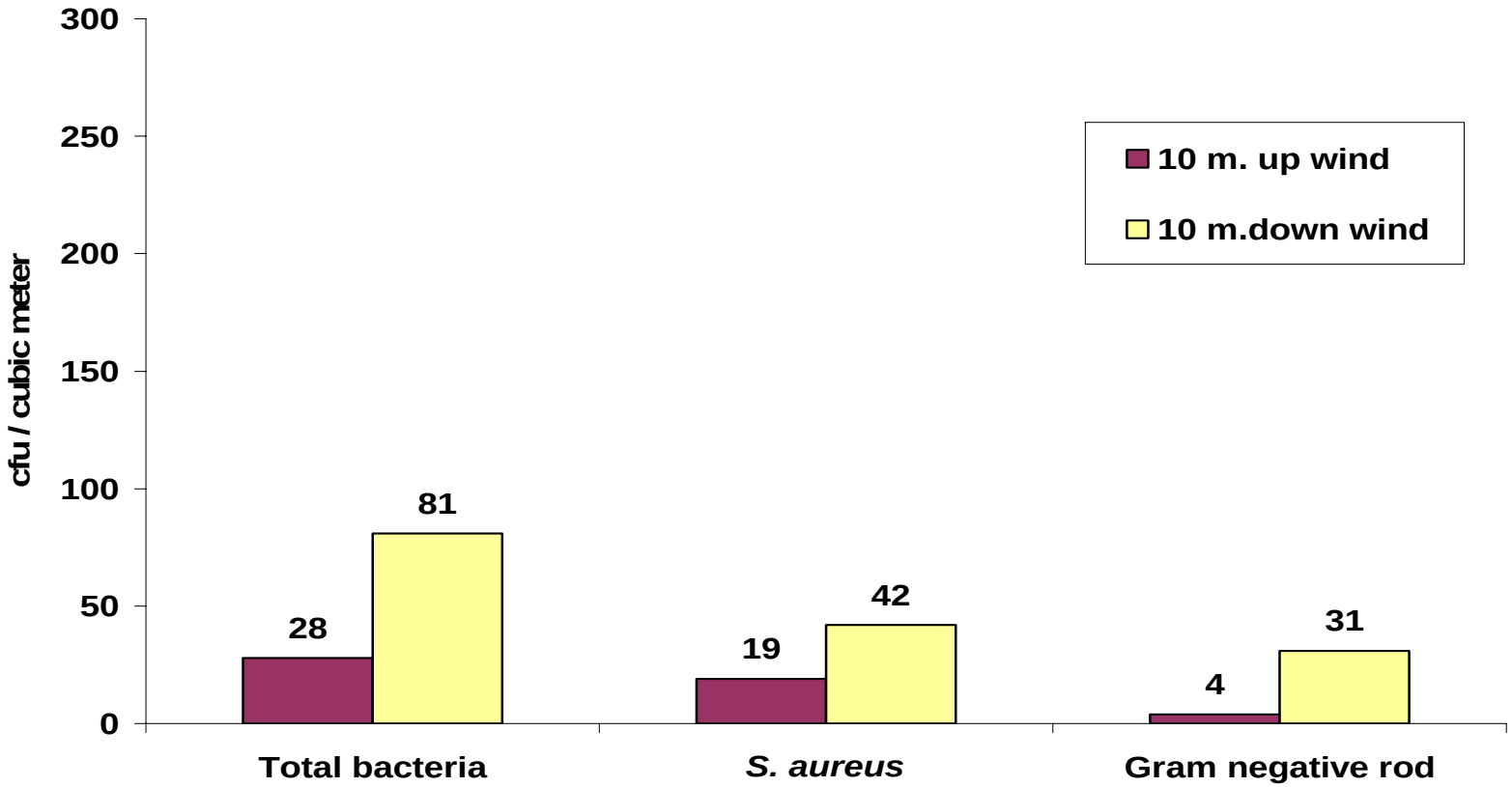

Figure AI-9.Bacteria collected in all plates together of Andersen's sampler on November 10, 2004 (During biosolids-application at Riker's field). The samples were collected from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature for this date was 12ºC with the wind from NNE- NE.

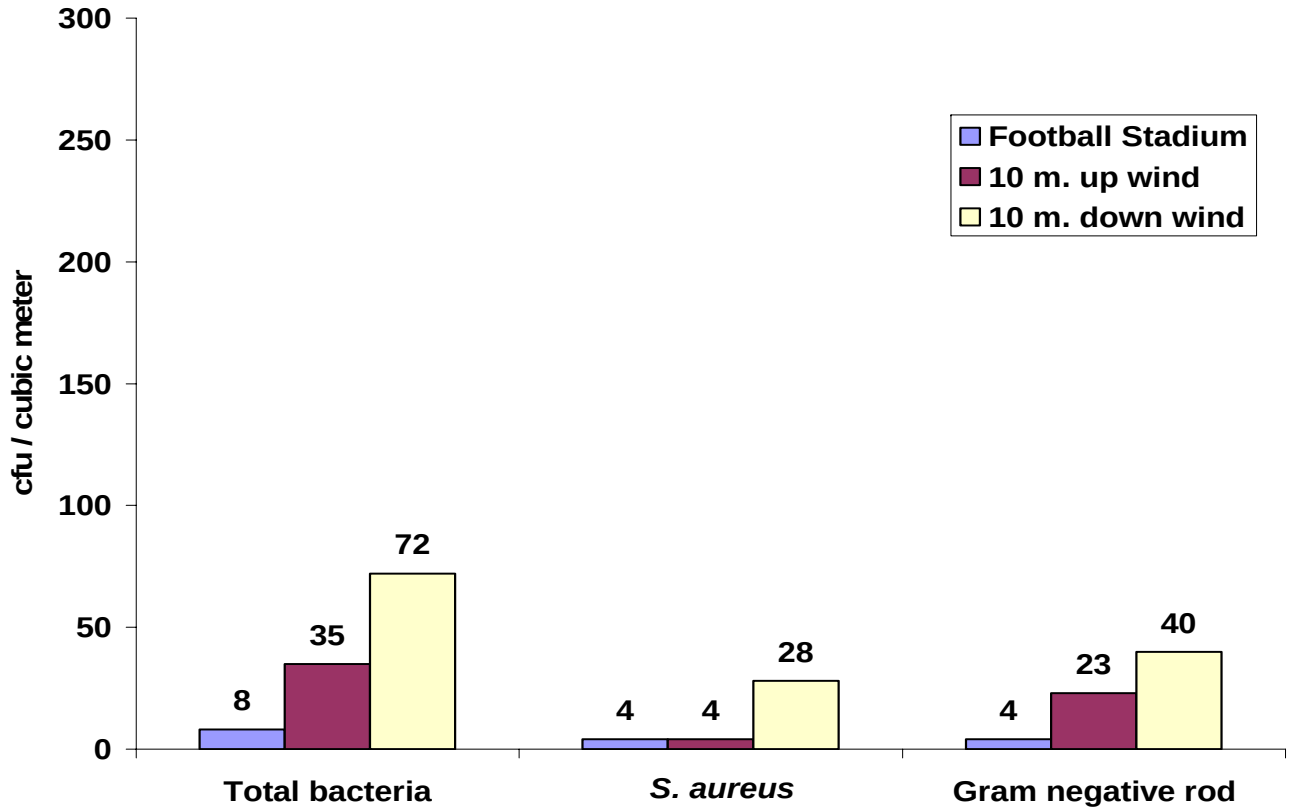

Figure AI-10.Bacteria collected in first and second plates together of Andersen's sampler on November 12, 2004 (Two days after biosolids-application at Riker's field). The samples were collected at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature for this date was 6.1ºC with the wind from NNE-NE.

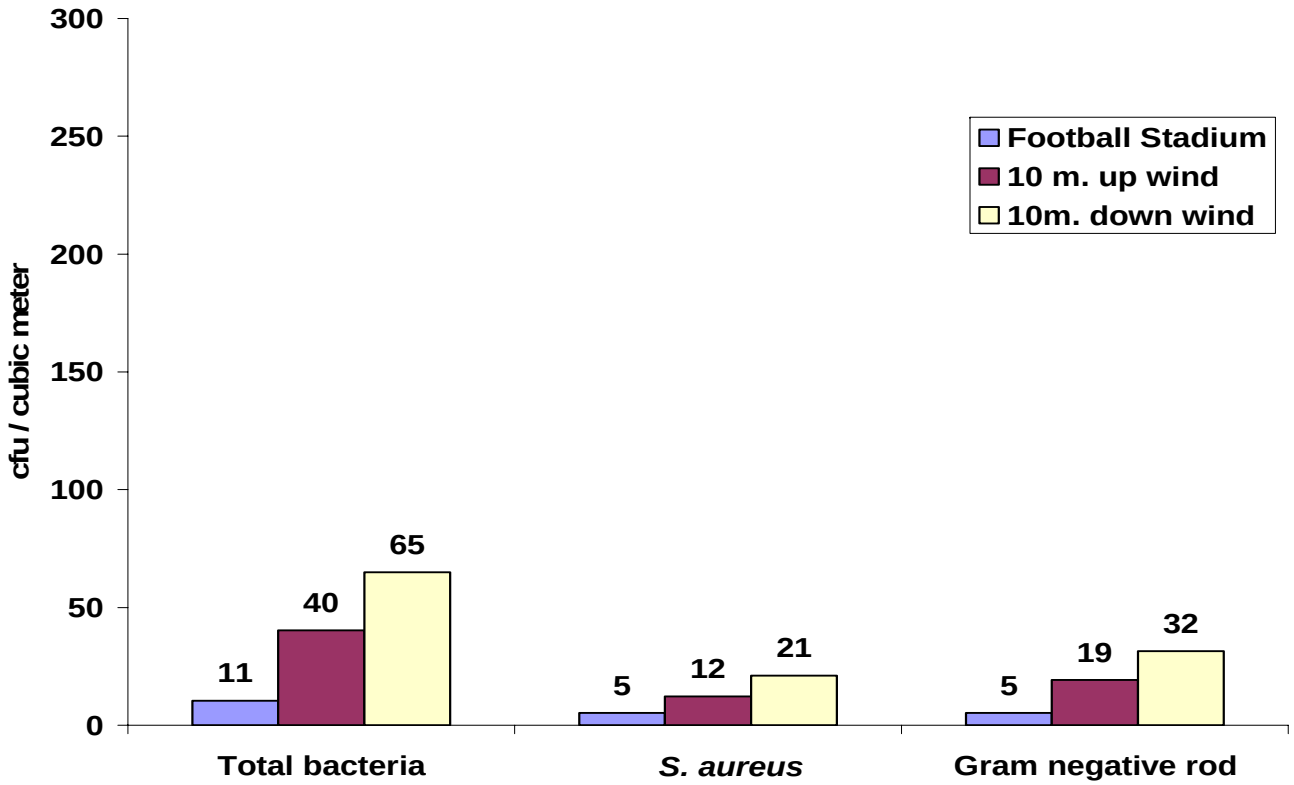

Figure AI-11.Bacteria collected in third and fourth plates together of Andersen's sampler on November 12, 2004, (Two days after biosolids-application at Riker's field). The samples were collected at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature for this date was 6.1ºC with the wind from NNE-NE.

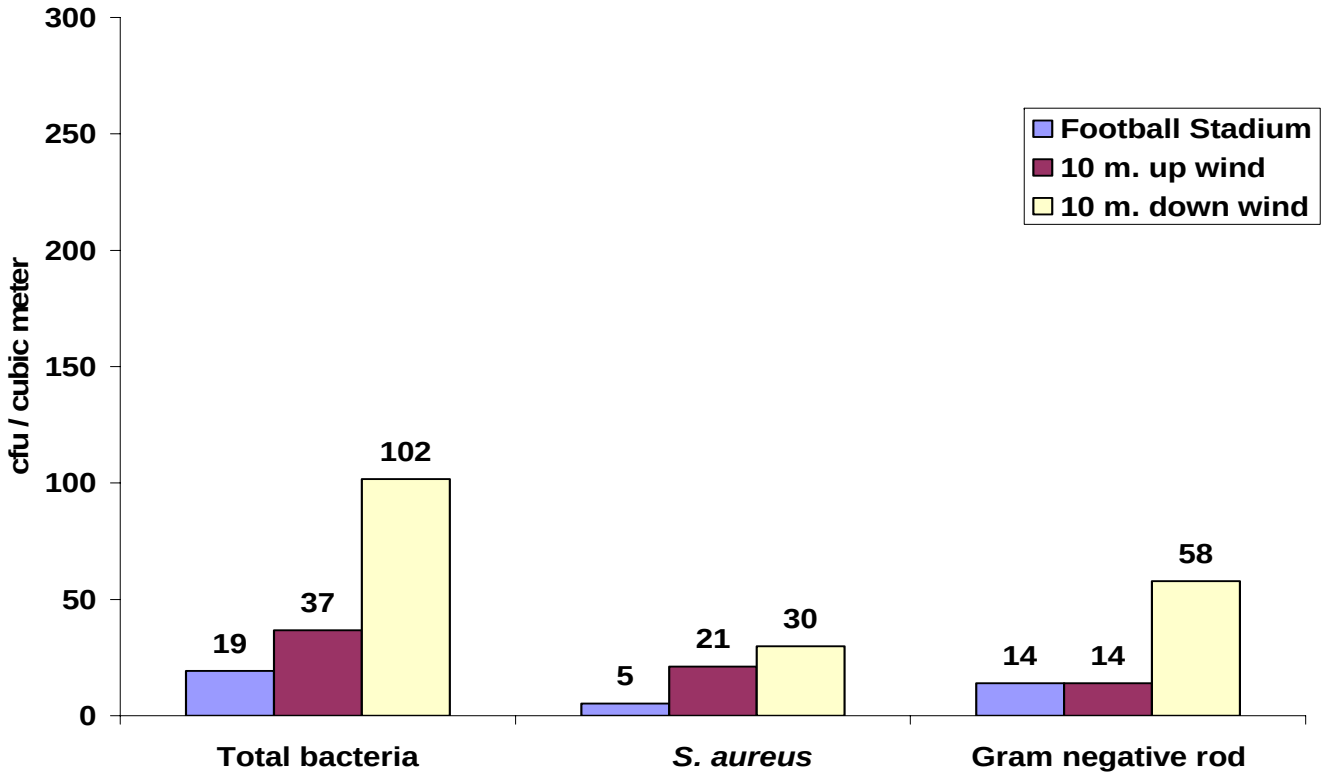

Figure AI-12.Bacteria collected in fifth and sixth plates together of Andersen's Sampler on November 12, 2004 (Two days after biosolids-application at Riker's field). The samples were collected at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature for this date was 6.1ºC with the wind from NNE-NE.

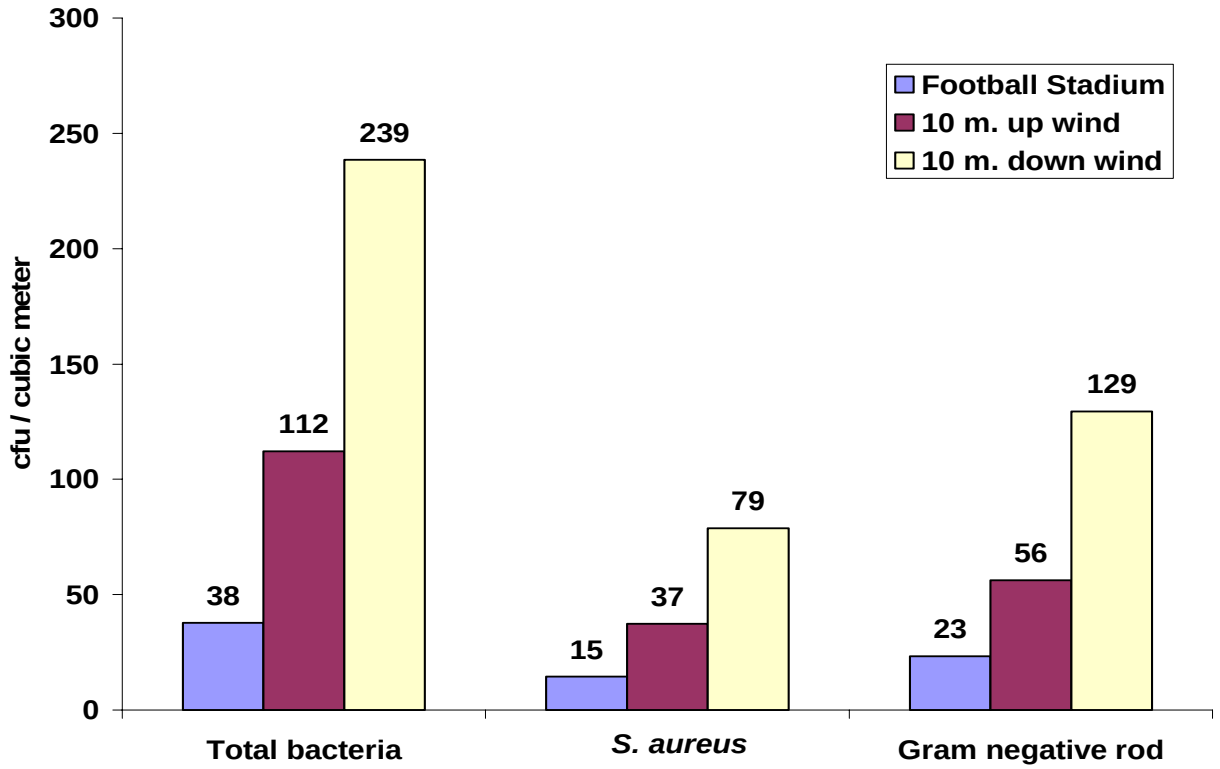

Figure AI-13.Bacteria collected in all plates together of Andersen's Sampler on November 12, 2004 (Two days after biosolids-application at Riker's field). The samples were collected at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature for this date was 6.1ºC with the wind from NNE-NE.

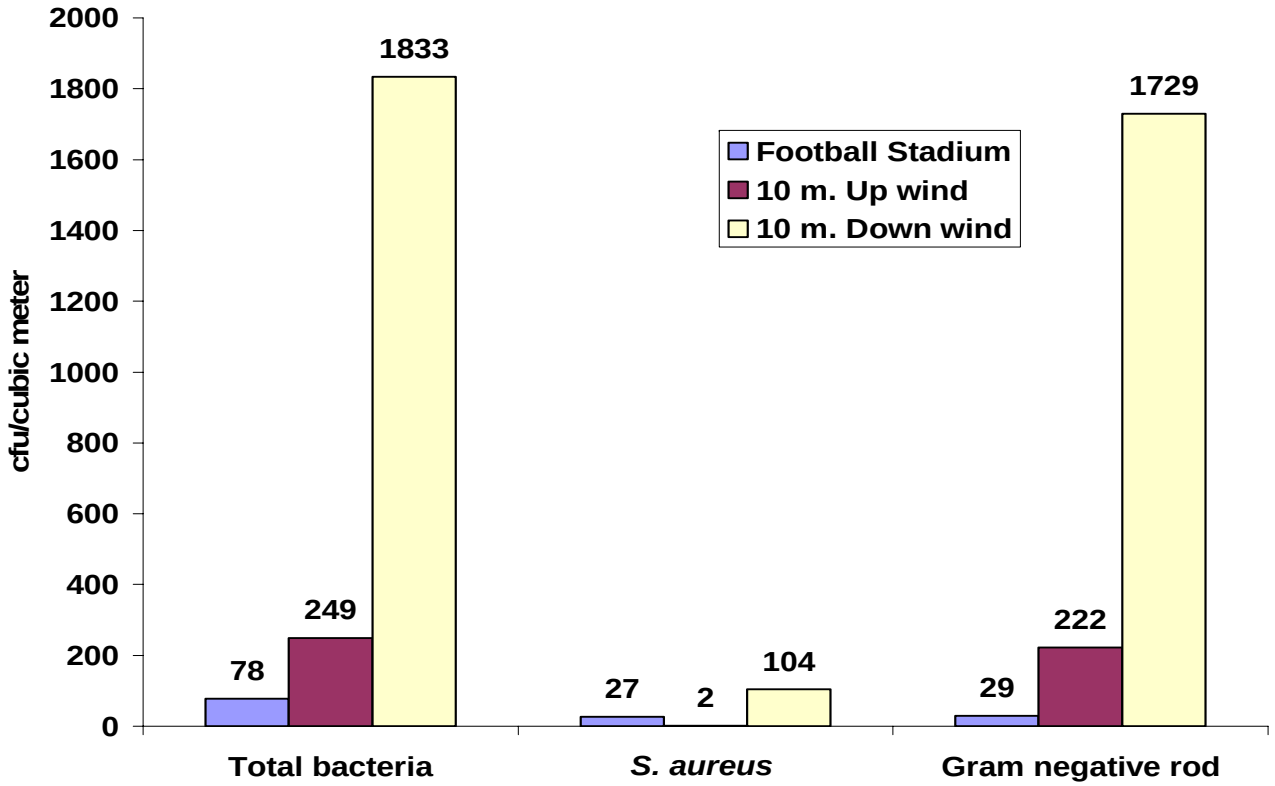

Figure AI-14.Bacteria collected in Microflow sampler (single stage sampler) on November 12, 2004 (Two days after biosolids-application at Riker's field). The samples were collected at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature for this date was 6.1ºC with the wind from NNE-NE.

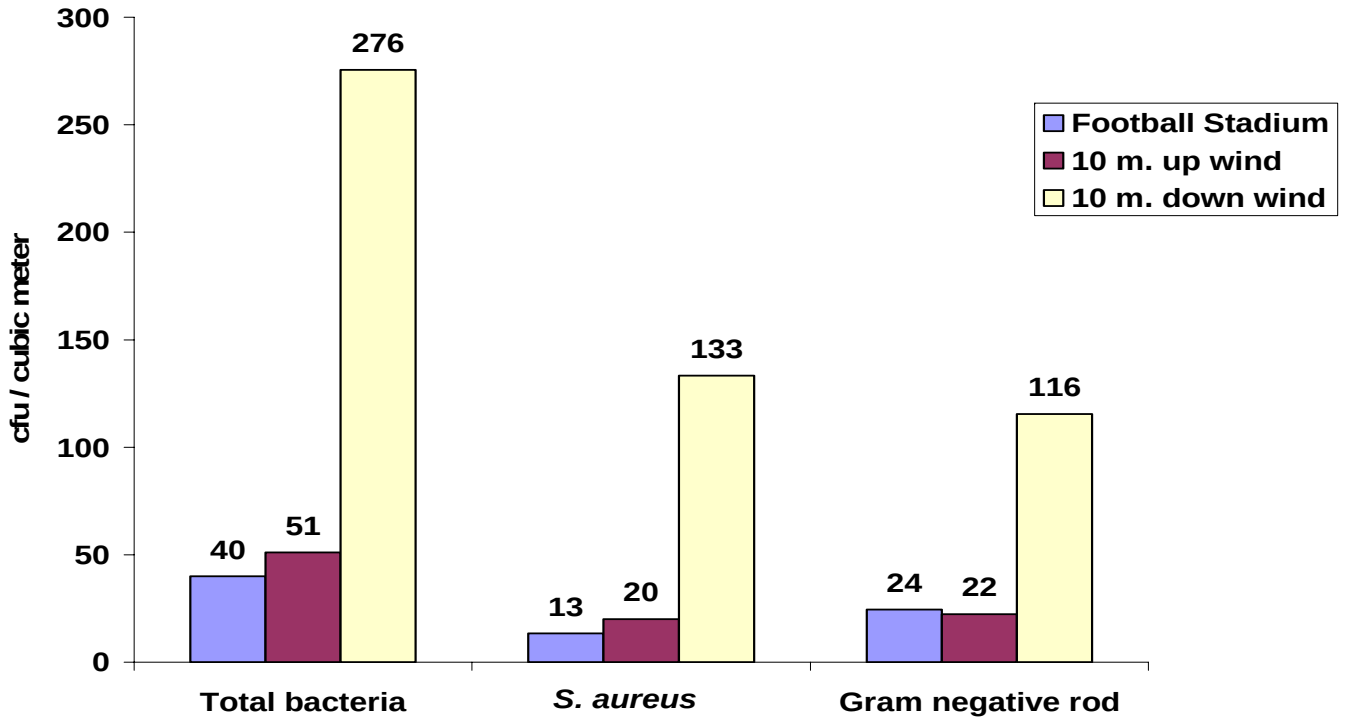

Figure AI-15.Bacteria collected in Microflow sampler (Single stage sampler) on November 23, 2004 (13 days after biosolids-application at Riker's field). The samples were collected at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at George Riker's field, Bowling Green, Ohio. The noon temperature for this particular date was 12ºC Noon with the wind from SSE.

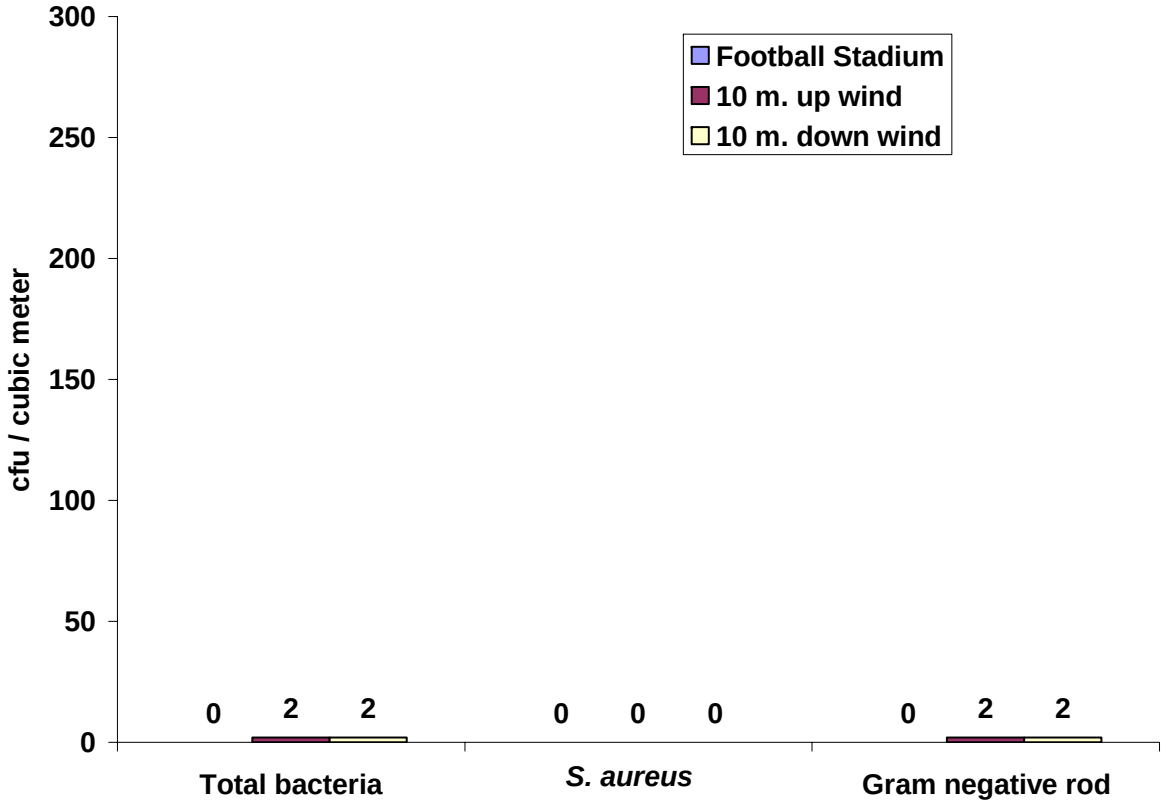

Figure AI-16.Bacteria collected in first and second plates together of Andersen's sampler on March 03, 2005 (Thirty days after biosolids-application at Bowling Green Corporation Field, Bowling Green, Ohio). The samples were collected at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at Bowling Green Corporation Field. The noon temperature for this particular date was 19.4ºC with the wind from SE.

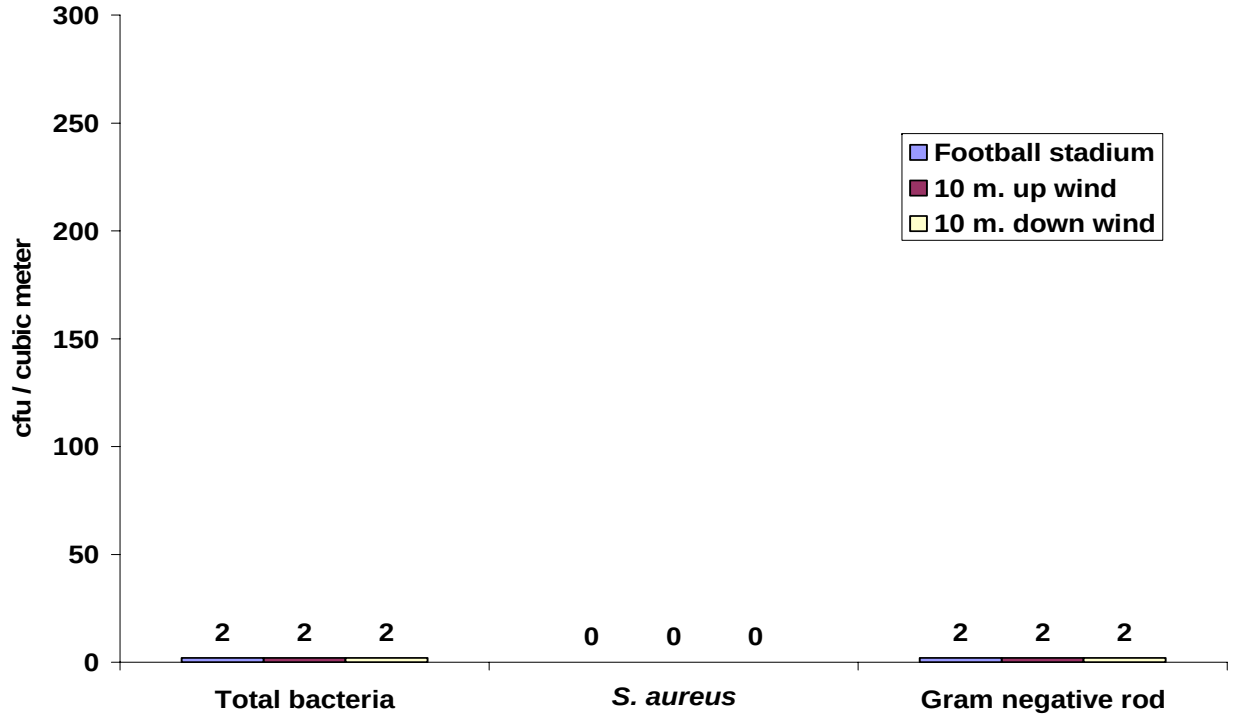

Figure AI-17.Bacteria collected in third and fourth plates together of Andersen's sampler on March 03, 2005, (Thirty days after biosolids-application at Bowling Green Corporation Field, Bowling Green, Ohio). The samples were collected at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at Bowling Green Corporation Field, Bowling Green, Ohio. The noon temperature for this particular date was 19.4ºC with the wind from SE.

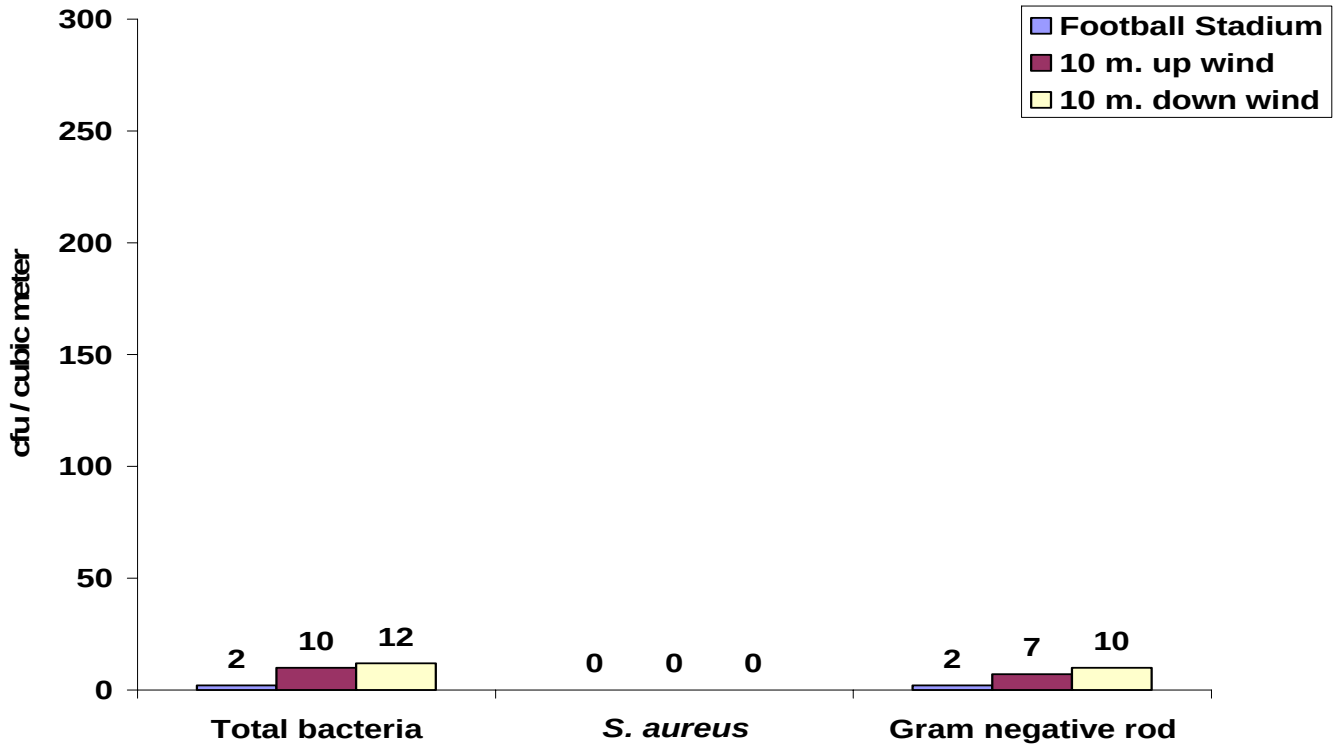

Figure AI-18.Bacteria collected in fifth and sixth plates together of Andersen's sampler on March 03, 2005 (Thirty days after biosolids-application at Bowling Green Corporation Field, Bowling Green, Ohio). The samples were collected at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at Bowling Green Corporation Field, Bowling Green, Ohio. The noon temperature for this particular date was 19.4ºC with the wind from SE.

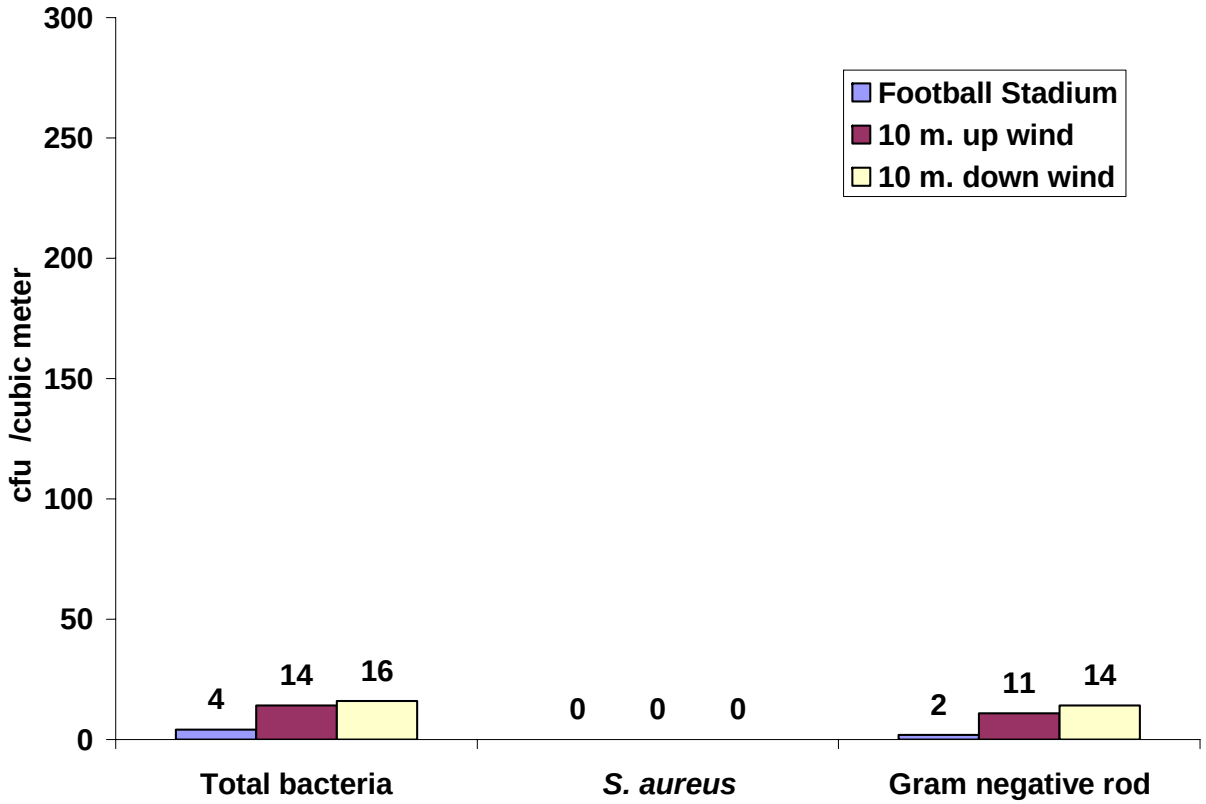

Figure AI-19.Bacteria collected in all plates together of Andersen's Sampler on March 03, 2005 (Thirty days after biosolids-application at Bowling Green Corporation Field, Bowling Green, Ohio). The samples were collected at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at Bowling Green Corporation Field, Bowling Green, Ohio. The noon temperature for this particular date was 19.4ºC with the wind from SE.

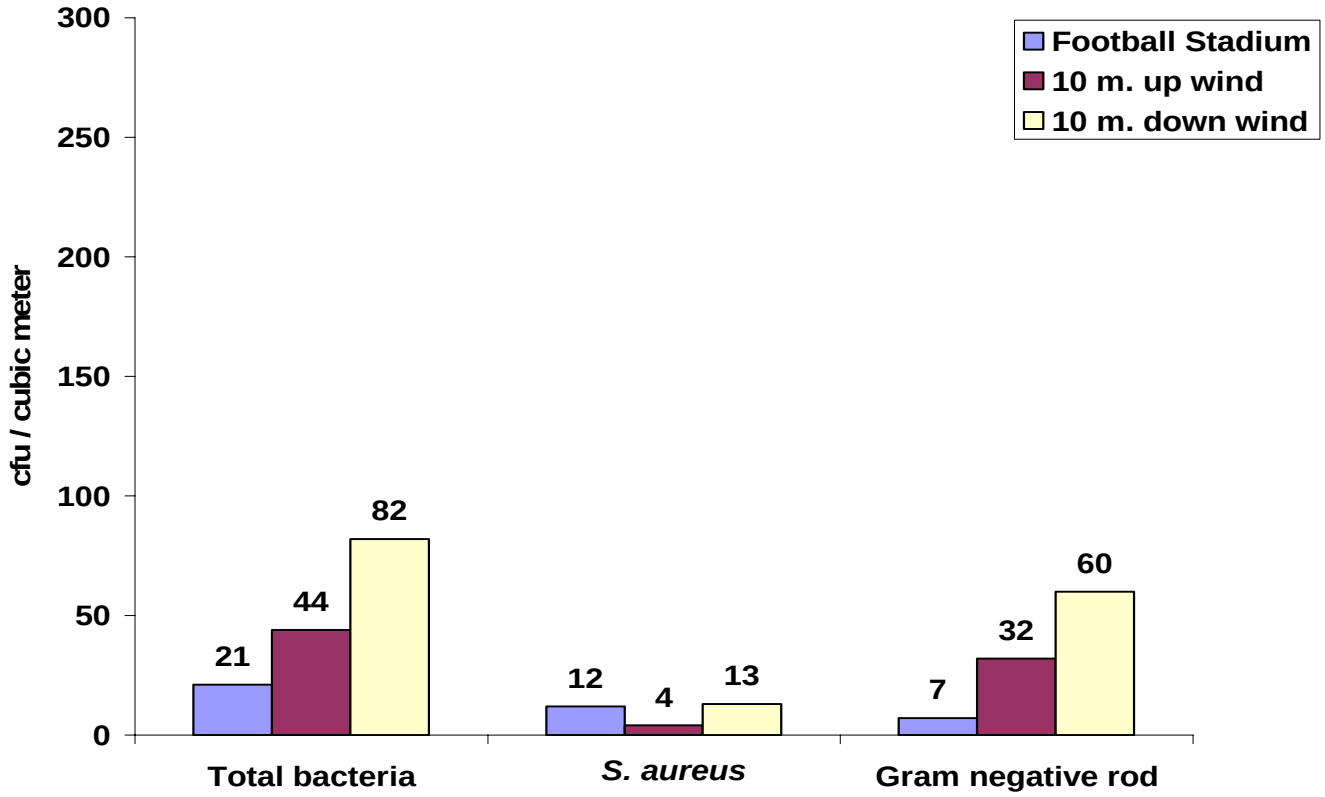

Figure AI-20.Bacteria collected in Microflow sampler on March 03, 2005 (Thirty days after biosolids-application at Bowling Green Corporation Field, Bowling Green, Ohio). The samples were collected at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at Bowling Green Corporation Field, Bowling Green, Ohio. The noon temperature for this particular date was 19.4ºC with the wind from SE.

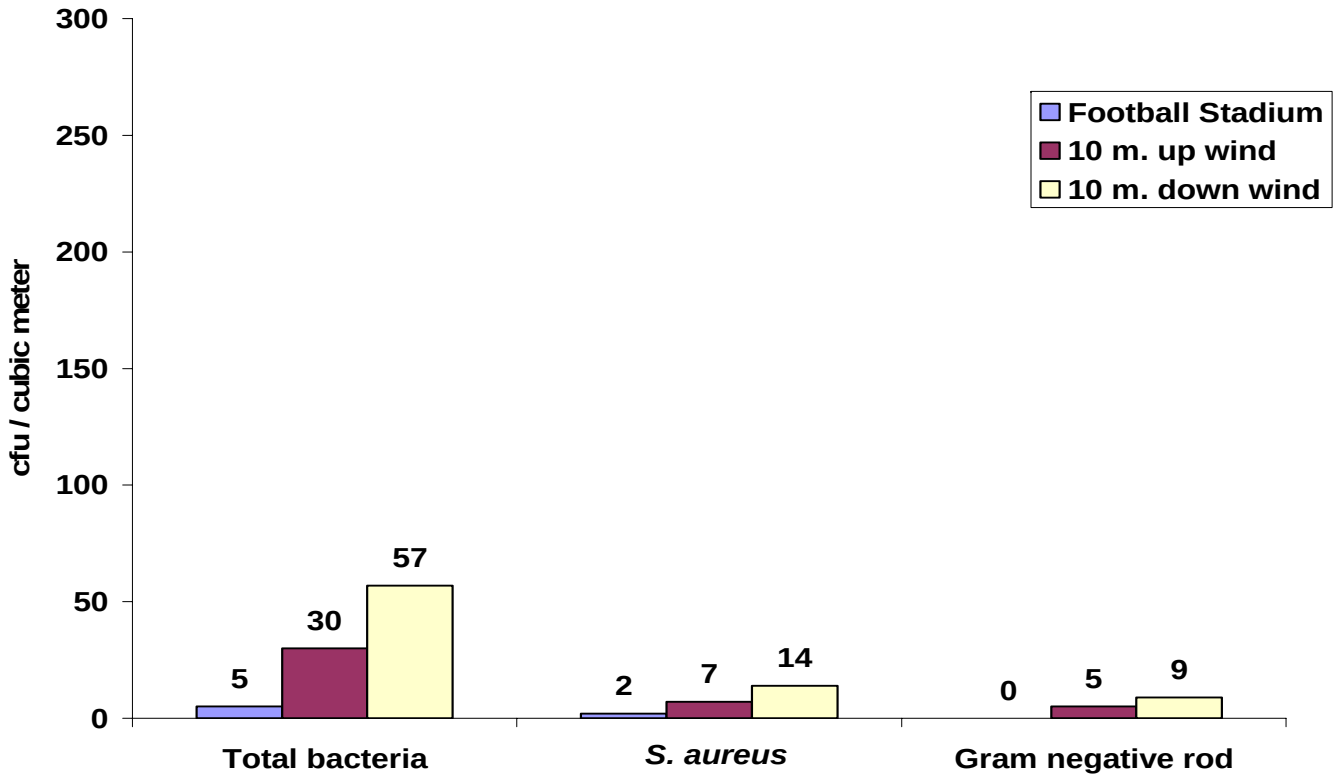

Figure AI-21.Bacteria collected in first and second plates together in Andersen's Sampler on March 03, 2005 at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at WWTP, Bowling Green, Ohio. The noon temperature for this particular date was 19.4ºC with the wind from SE.

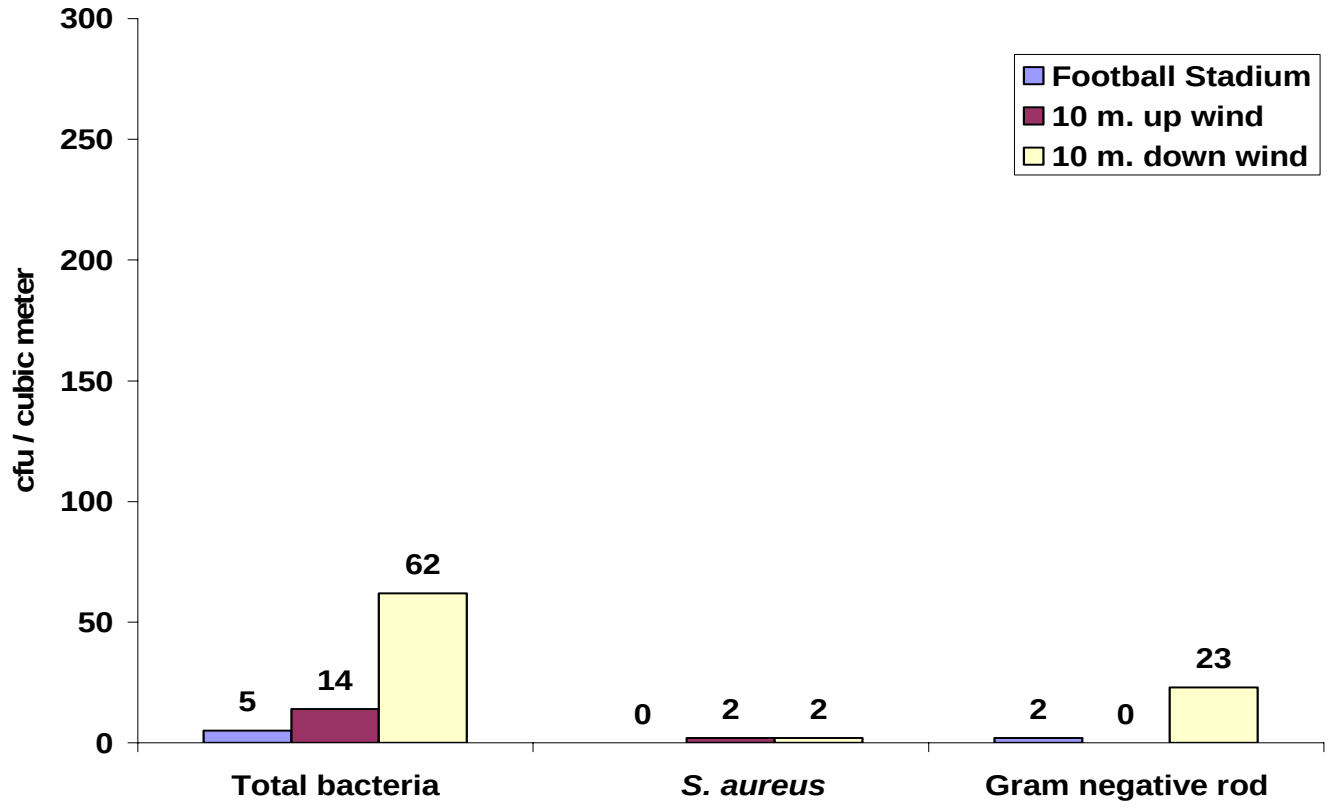

Figure AI-22.Bacteria collected in third and fourth plates together in Andersen's Sampler on March 03, 2005 at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at WWTP, Bowling Green, Ohio. The noon temperature for this particular date was 19.4ºC with the wind from SE.

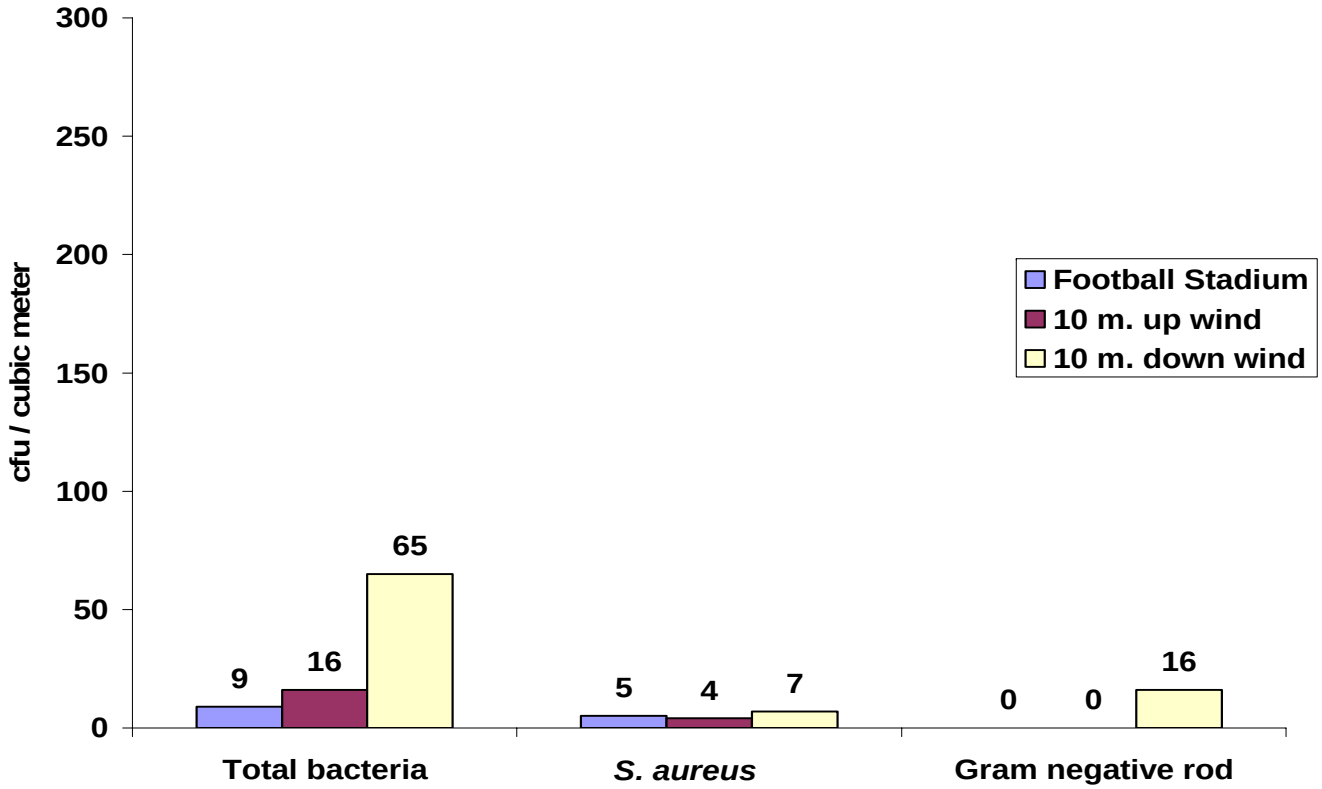

Figure AI-23.Bacteria collected in fifth and sixth plates together in Andersen's Sampler on March 03, 2005 at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at WWTP, Bowling Green, Ohio. The noon temperature for this particular date was 19.4ºC with the wind from SE.

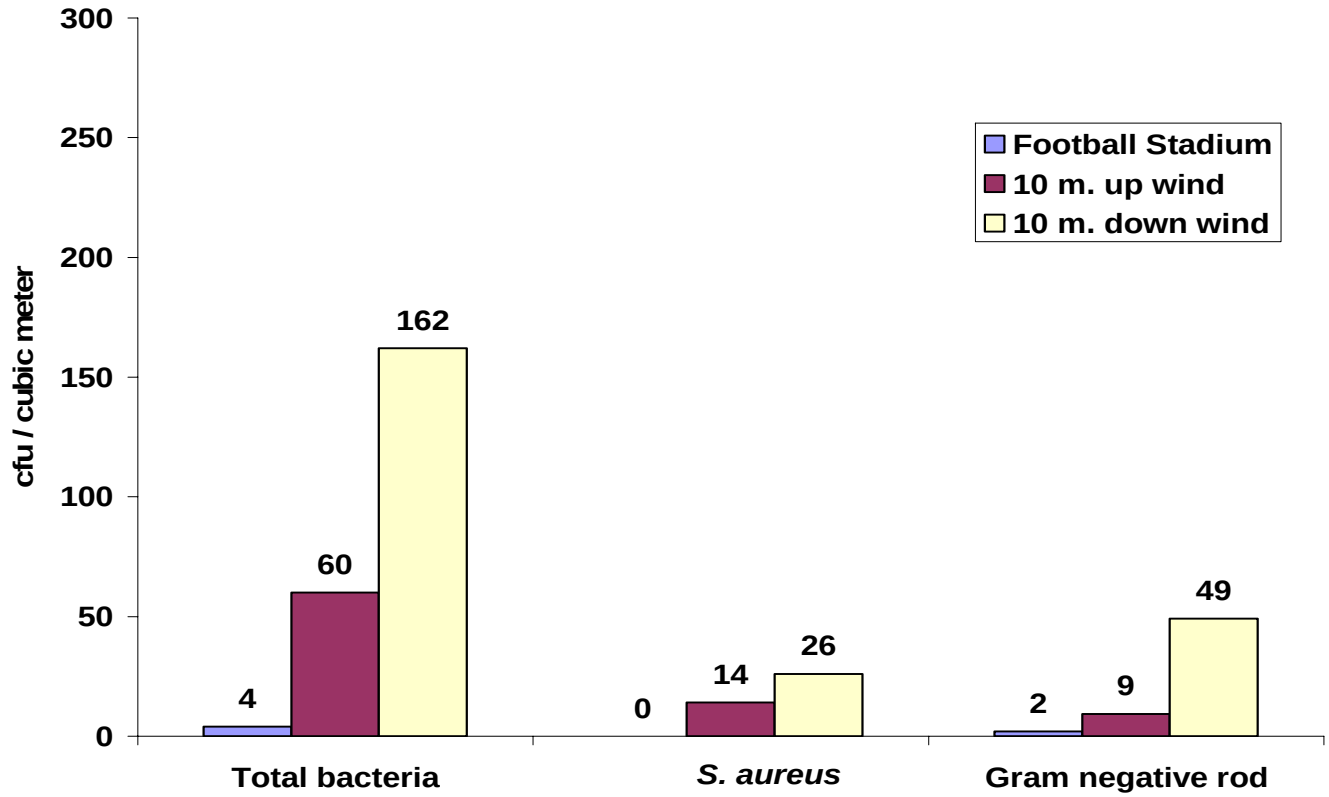

Figure AI-24.Bacteria collected in all plates together in Andersen's Sampler on March 03, 2005 at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at WWTP, Bowling Green, Ohio. The noon temperature for this particular date was 19.4ºC with the wind from SE.

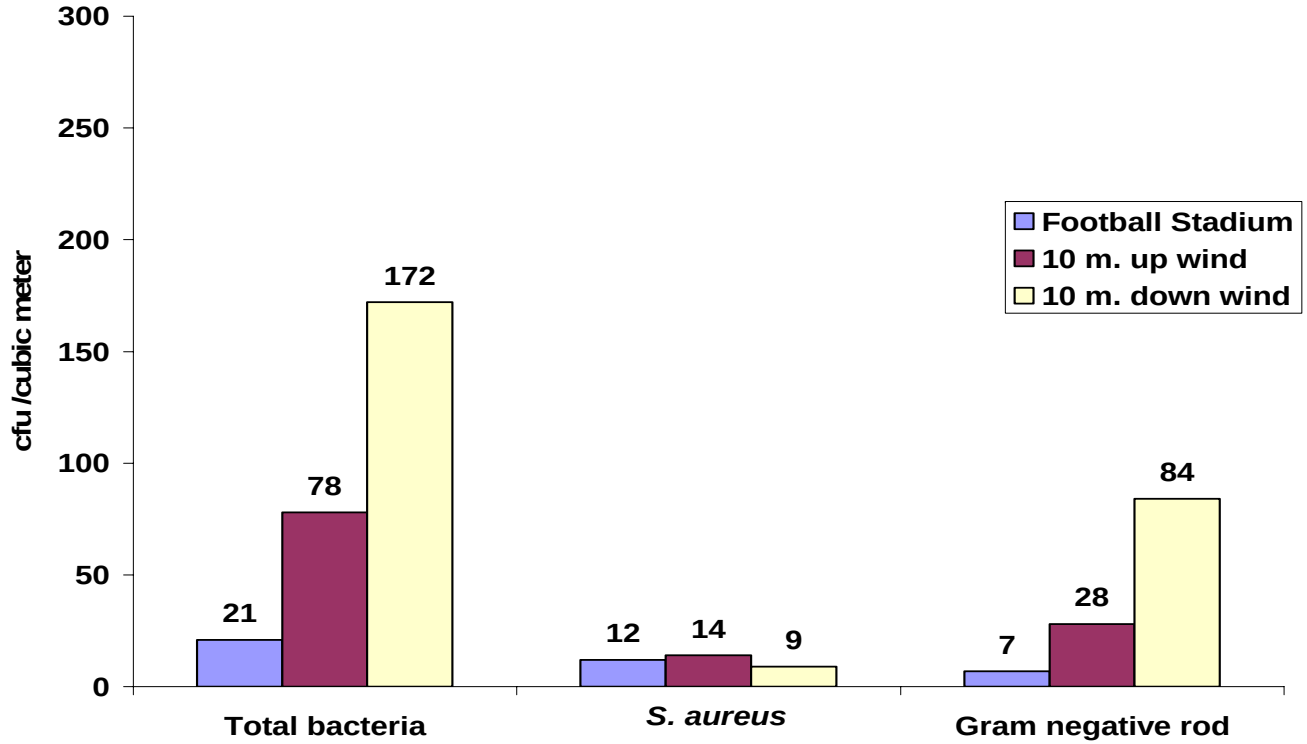

Figure AI-25.Bacteria collected in Microflow sampler (Single stage sampler) on March 03, 2005 at the Football stadium, Bowling Green, Ohio and from the upwind and downwind directions at Bowling Green WWTP, Bowling Green, Ohio. The noon temperature for this particular date was 19.4ºC with the wind from SE.