Study IIe

Studies of Fungal Spore Dispersion from Confined Animal Feeding Operations (CAFOs) in FY12

for the

Assessment of Land-based Sources of Air Quality Contaminants in the Binational Border Region of Southwestern New Mexico, Northwestern Chihuahua and West Texas

Prepared for the Department of Health
Office of Border Health
1170 N. Solano Dr.
Las Cruces, NM 88001

Submitted by

Principal Investigators

Dr. Dave DuBois
Erin Ward

Additional Contributors:

Dr. Soum Sanogo
Melanie Lytle
Elizabeth Smith
Rebecca Armenta

New Mexico State University
Las Cruces, NM 88003

June 30, 2012

ACKNOWLEDGEMENTS

This project is being carried out by New Mexico State University in association with the University of Texas El Paso, Autonomous University of Juarez (Chihuahua), the Desert Research Institute, and the University of Arkansas for Medical Sciences.

This work is being funded under a MOA 13828 with the New Mexico Department of Health, Office of Border Health.

Mr. Paul Dulin provided overall project management.

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1. Introduction

1.1. Background

While total particulate matter mass concentration has been the primary indicator in epidemiological studies, a positive correlation with measurable health effects may be the result of specific chemicals or the composite of several species within the aerosols. One of the main problems is the fact that ambient particulates are composed of many substances, with some unidentified, and not all of the components have equal toxicity. Co-pollutants may also confound the study making it inconclusive in terms of identifying the causes of increased hospital visits or mortality. Highly prevalent in urban aerosols, organic compounds formed from the condensation of volatile organic vapors may be a pathway for causing health effects by acting as irritants or allergens. Elemental carbon aerosols, from combustion processes, have been hypothesized as transporters of organic and inorganic compounds. Particles with reactive-transition metals such as Vn, Cu, Fe and Pl are known to have toxic and inflammatory properties (Mauderly et al., 1998). Other chemical species that may have negative health effects include peroxides. Bioaerosols have largely been ignored in health effects studies and are beginning to be studied as a possible pathway for health effects. The bioaerosols include pollen, viruses, bacteria, and microbes such as spores and fungi (Cox and Wathes, 1995).

In this study we characterize the bioaerosol emissions from livestock feeding areas where numerous animals are present. The US EPA classifies these as either animal feeding operations (AFOs) or concentrated animal feeding operation or CAFOs depending on how long the animals are present at the facility. If they keep animals confined to a small area for at least 45 days over a 12-month period they are classified as an AFO by the regulations. A dairy that allows for animals to roam in a pasture is not technically an AFO. A CAFO designation is given if the AFO allows for wastewater to leave the facility (discharge) into the surroundings or into natural waters. Those that fit the category for CAFO are required to have a federal National Pollutant Discharge Elimination System (NPDES) water quality permit. Due to the effects of dispersion and gravitational settling, the impacts of a CAFO on bacterial concentrations in the local can be detected within 200 meters of the facility (Green et al., 2006; Dungan et al., 2010) during low wind situations. High wind events can loft particles high in the atmosphere and disperse them great distances from the CAFO.

The characterization involves the measurement of particulate chemical, elemental, and biological composition from these facilities as well as the surrounding area over time. This year we initiated our data collection in southern New Mexico to assess the fungal composition of bioaerosols at several locations including Columbus, Palomas (Mexico), Sunland Park, and Las

Cruces. Additional locations were in northwest El Paso, Texas and the area around Vado and Berino, NM. Similar studies in El Paso and Doña Ana County have noted that the highest indoor bioaerosol concentrations occur in the winter, contradicting other studies carried out in other parts of the US (Mota et al., 2008). However, they did observe that the outdoor and indoor bioaerosol concentration correlated in all seasons except winter. Alverado et al. (2009) found out that fungal, bacterial, and S. aureus were most prevalent during the Spring at a location within a 12,500 cattle dairy in Doña Ana County.

1.2. Study IIe Objectives

The objectives of this study are:

- Build a taxonomic database of bioaerosols in an near animal feeding operations within the Binational Border Region of Southwestern New Mexico, Northwestern Chihuahua and West Texas
- To acquire a database of environmental conditions in and around the study area that are relevant to studies of soil fungi

2. Ambient Measurements

2.1. Monitoring Strategy

At each location, a standard operating protocol (Figure 1.1-1) was followed to assess the fungal composition of air. The following protocol included:

1. Expose 9-cm diameter plastic Petri plates containing growth media (e.g. acidified potato dextrose agar, APDA) to the outdoor air
2. Remove plates after desired duration
3. Place Petri plates at room temperature (22-25°C)
4. Monitor Petri plates for fungal growth
5. Enumerate fungal colonies
6. Catalogue and enumerate each fungal colony based on color, growth pattern, and texture

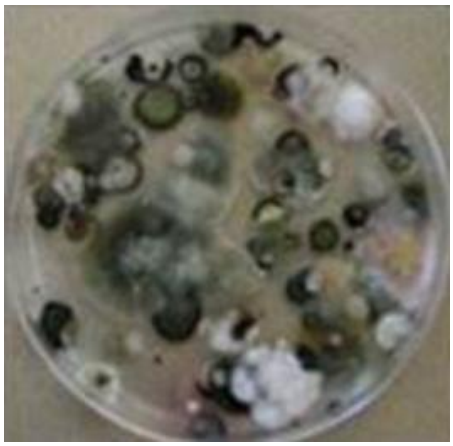


Figure 2.1-1. Example of fungal colony from air sample

7. Establish pure culture of each type of fungal colony on potato dextrose agar (PDA) or APDA
    a. Characterize each colony type by molecular method through PCR
    b. Examine each colony type by microscopy

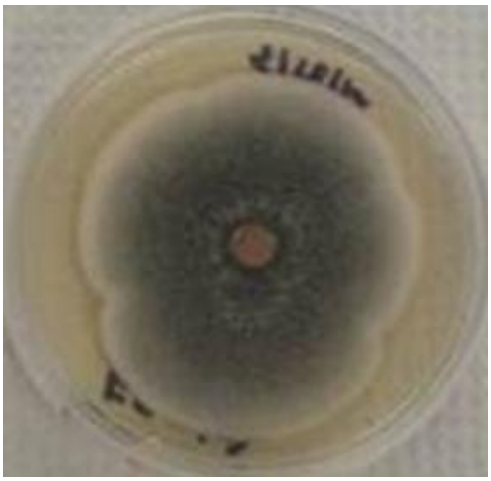


Figure 2.1-2. Example pure culture on a plate

2.2. Petri Plate Sampling

Petri plates containing acidified potato dextrose agar medium were exposed for duration from 1 to 3 hours and time periods between 9 am and 9 pm. Following exposure, the Petri plates were maintained at room temperature (22 - 25°C) for 5 to 7 days on a laboratory bench. Emerging fungal colonies were enumerated and catalogued in groups of similar characteristics based on morphological attributes.

2.3. Samples Collected

2.3.1. Columbus and Palomas Cattle Yards

An effort was made to collect fungal samples at the Union Ganadera in Palomas, Mexico and at the adjacent Luna County Cattle yard in Columbus, New Mexico. The data collection was part of a short-term PM_{10} characterization and monitoring network in Palomas in the Spring of 2012. The focus of the PM_{10} and meteorological measurement network was on collecting data to help understand the impacts of fugitive dust sources such as the cattle facility and unpaved roads in and around Palomas. To do this we installed and operated a small network of aerosol samplers surrounding the Union Ganadera cattle facility (Figure 2.3.1-1) over a period of a month. Sample collection began on March 28, 2012 and end on April 29, 2012.

Figure 2.3.1-1. Union Ganadera viewed from the west side (looking toward the east). The far left is the international border fence with light poles. Note that residential houses are approximately 10 meters from this fence.

2.3.2. Thunderstorm Outflow Winds

Two samples that were collected based on the environmental conditions were May 21 and June 15, 2011 in Las Cruces. These two samples represent transported dust particles during a dry thunderstorm outflow event. Thunderstorm outflow events occur when thunderstorm downdrafts within the main storm cloud system exit, impact the earth's surface and flow outward away from the storm. In some cases the outflow flows in all directions and other times it flows in one direction depending on surrounding synoptic winds. As the outflow winds push away from the storm cloud, it brings with dust, insects, and aerosols that were lofted from the

surface along the way. On May 21 the outflow winds moved westward (Figure 2.2.2-1), advecting dust, insects, and bioaerosols from the east side of town toward the west side. The yellow lines indicate the general direction of the outflow boundary. The thunderstorm that generated the outflow was located over southeast Otero and northern Hudspeth counties.

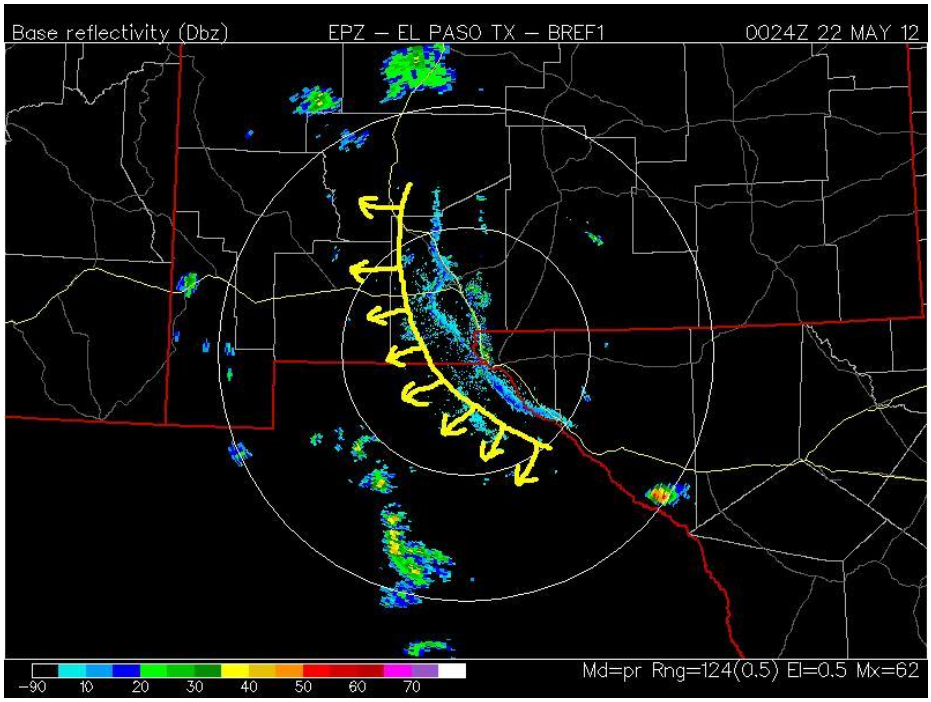


Figure 2.3.2-1. Outflow boundary moving toward the west on May 23, 2012. Boundary shows up as the bluish trace on the radar plot.

The June 15 event was similar but the outflow boundary was not as distinct although it generated a haboob dust event in El Paso and along the Organ Mountains that evening.

2.3.3. Dust Control Demonstration Plots

We obtained fungal samples from the first dust control plot on May 25 after the controls were in place. One sample was on the uncontrolled plot and the other was on a polymer treated plot. The plots are oriented with the longest dimension (100 feet) running east to west. Winds were from the west southwest on that day so some particles saltating on the test plot were very likely captured on the Petri plate over the 2 hours exposure.

Fungal samples were collected at the Diamond Springs dust control plot on June 15. This collection was also during a thunderstorm outflow event as described in section 2.3.2. In both cases the Petri plate was place directly on the ground to maximize the collection of larger sand particles that come from the immediate dust control surface.

2.3.4. Dairy Samples

We have noticed that there are specific times that the animal feedling operation generates more particulate emissions than others. Peak emissions occur in the late afternoon and early evening and has been noted by others with anecdotal evidence and during emission studies (Sweenten et al., 1998; Guo, 2011; Hamm, 2005) and attributed to increased animal activity. The sampling strategy is to obtain grab samples from the frontage road or other public roads near the dairies between Mesquite and Berino, New Mexico. This can be done from a vehicle moving slowly in the 30 to 40 mph range (only if safe to drive that speed) along the road holding out a Petri plate. The slower the better since small particle impaction is severely limited when wind velocity is high. The simple method is to hold it out the window so the wind hits the agar side as the car passes the cattle yard. To insure enough sample, we tried to get at least two passes over the same area. Exposure time was typically a minute or two depending on speed. These samples have been exposed at the end June and are in the process of being grown in the laboratory and not available in time for this report.

Table 2.3.4-1 shows the status of samples collected for the project and information on the environmental conditions surrounding the sampling time.

Table 2.3.4-1. Sampling locations where Petri plates were exposed outdoor to assess fungal composition of air

DATE	City	LOCATION/SOURCE	Height above ground (m)	WD, WS (m/s), T(C)	ABBREVIATION FOR LOCATION	TIME OF EXPOSURE	TOTAL # OF FUNGI	Notes
5/1/2012	Palomas, MX	West Side of cattle yard-Palomas; placed on top of blow wall	2	W, 3, 28	WS	12:00pm-2:00pm	10	
5/1/2012	Palomas, MX	East Entrance-Palomas; placed on top of block wall	2	W, 3, 28	EE	12:00pm-2:00pm	21	
5/17/2012	Columbus, MX	East end of Luna County cattle yard; placed on fence in parking lot	1	VAR, 1.5, 27	COL-EAST	9:45 am-12:53 pm	17	calm winds
5/17/2012	Columbus, MX	West end of Luna County cattle yard; placed within cattle holding area on a fence post	2	VAR, 1.5, 27	COL-WEST	9:48 am-12:55 pm	18	calm winds
5/18/2012	Sunland Park, NM	at Sunland Park Casino parking lot	1	SW, 4, 28	SLP	7:47 pm-8:55 pm	20	
5/18/2012	El Paso, TX	northwest El Paso, near Artcraft exit on I-10	1	WSW, 4.5, 26	ELP	9:38 pm-11:54 pm	7	night sample
5/21/2012	Las Cruces, NM	NMSU (Weather station)	0.5	SE, 2.2, 30	NMSU-WX-STN	6:16 pm-8:13 pm	53	Brief T-storm outflow winds from east, strong gusty winds
5/22/2012	Las Cruces, NM	NMSU campus	0.5	W, 2.7, 32	NMSU	6:15 pm-8:20 pm	40	elevated smoke plume from Whitewater-Baldy fire; very little smoke on ground
5/24/2012	Las Cruces, NM	Dust control demonstraton site at Colimas; Untreated section-Sonoma Ranch	0	WSW, 9, 28	UNT	10:05 am-12:30 pm	11	
5/24/2012	Las Cruces, NM	Dust control demonstration site at Colimas; Polymer-Sonoma Ranch Blvd	0	WSW, 9, 28	POLY	10:05 am-12:30 pm	8	
5/29/2012	Las Cruces, NM	NMSU	1.5	WNW, 3.6, 30	N529	9:30 am-11:30 am	11	
5/31/2012	Las Cruces, NM	NMSU	1.5	WSW, 2.4, 35	N531	2:30 pm-4:30 pm	14	
6/1/2012	Las Cruces, NM	NMSU Ground	1.5	S, 2.2, 35	NMSU-Ground	11:00 am-1:00 pm	34	smoke impacts from Whitewater-Baldy fire
6/15/2012	Las Cruces, NM	Dust control demonstration site at Diamond Springs, Site 3 (3rd plot starting from south)	0	S, 2.4, 34	DSS3	6:48 pm-8:38 pm	44	T-storm outflow from south
6/15/2012	Las Cruces, NM	Front yard of house on High Range; exposed to the east	0	S, 2.4, 34	HR	6:58 pm-8:49 pm	47	T-storm outflow from south

3. Database and Data Validation

The purpose of the data evaluation is to summarize the accuracy and precision of the measurements, to identify and investigate extreme and inconsistent values, and to perform data comparisons and investigate discrepancies in the data. Data to be used in this study will acquired from ongoing measurement efforts and those specific to this study.

For example we used meteorological data from the project weather stations to document fungal sample conditions. This data will need to be evaluated before use in the fungal data analysis. An evaluation is critical since experience has shown that even the best designed field studies contain errors that need to be found, quantified, and flagged. The evaluation will involve plotting and examining pollutant time series data to identify spikes and outliers for investigation. The evaluation will also consider comparisons between measurements of the same or similar variable at the same or nearby site using different measurement devices and procedures. We will use scatter plots, linear regression, and correlation analysis to do these comparisons.

4. Temporal and Spatial Variations in Fungal Species

Figure 4-1 shows an example of emerging fungal colonies on a petri plate that was exposed in May 2012 in Sunland Park, NM.

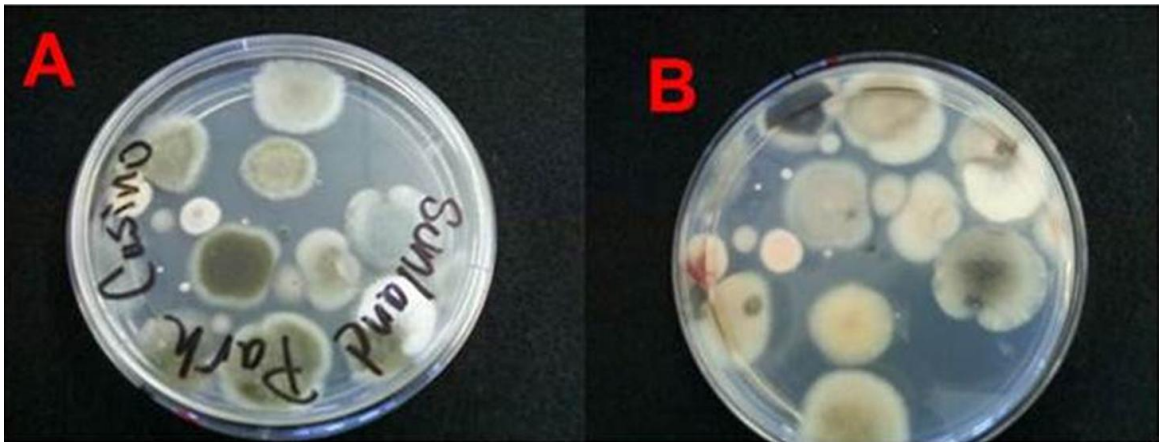
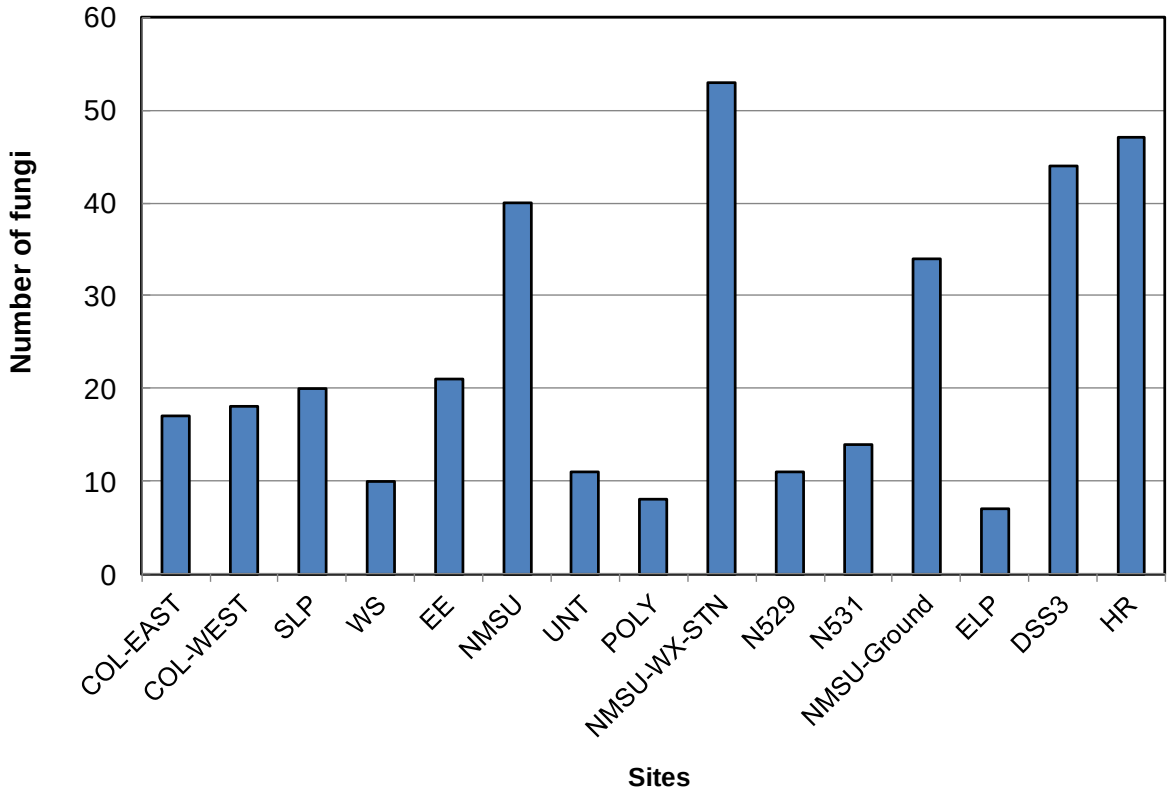


Figure 4-1. Fungal colonies on acidified potato dextrose agar in a Petri plate exposed to outdoor air at the Sunland Park Casino on May 18, 2012 from 7:47 to 8:55 pm. Top view and bottom view of the same Petri plate are shown in A and B, respectively.

Over all sites, the total number of fungi varied from 7 to over 50 as Figure 4-2 shows. The sample with the most number of fungi present was from the NMSU on May 21, 2012 during the thunderstorm outflow. Interestingly, several samples with the relatively highest number of fungal colonies are during thunderstorms. This will be investigated further in FY13 while we collect samples during the summer monsoon period. It will be interesting to see the effect of high soil moisture content once precipitation (hopefully) is more regular.



complete description of the sites is shown in Table 2.3.4-1.

Currently, fungal groups catalogued for each location are being identified using both morphological (color, growth pattern, texture, type of spores produced) and molecular approaches through polymerase chain reaction (PCR) technique using universal primers and species-specific primers. Preliminary results indicate that fungal colonies, grown from deposited dust particles, are diverse (Figure 4-3).

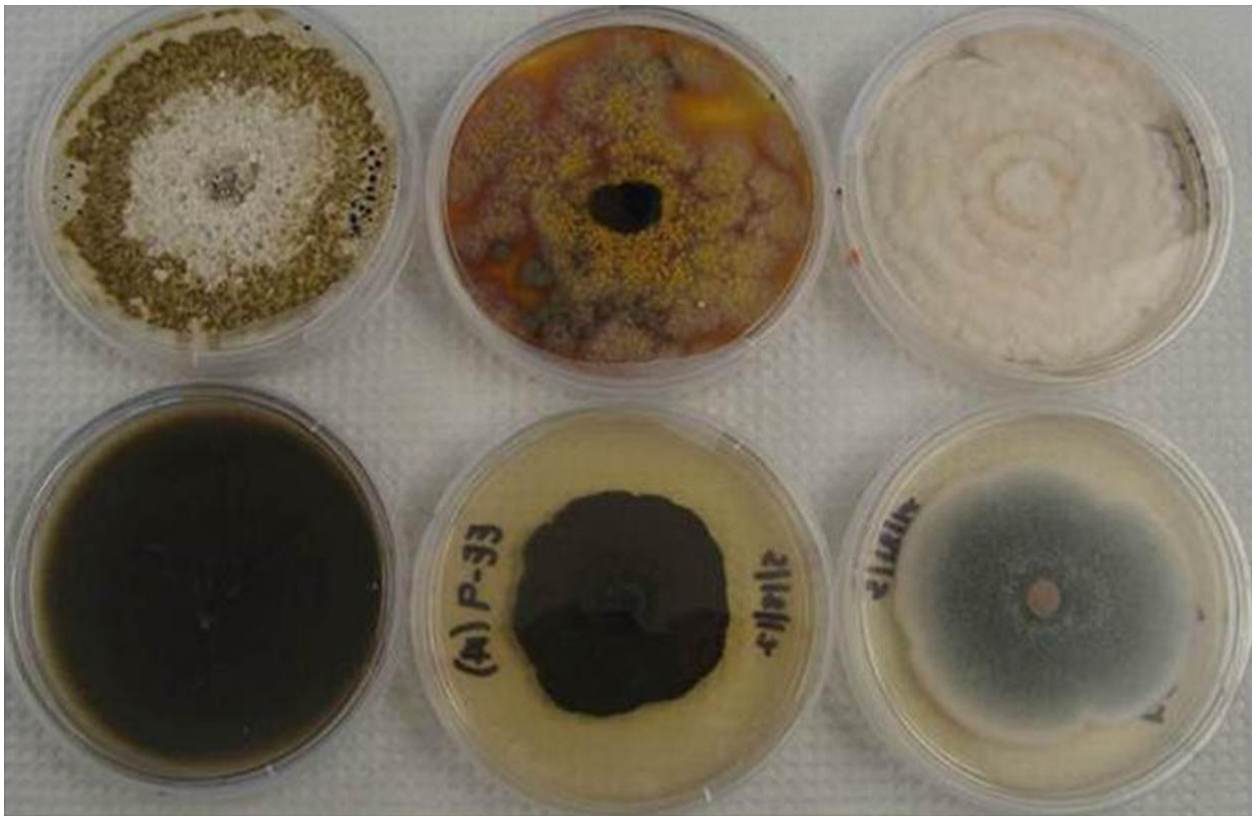


Figure 4-3. Examples of fungal microorganisms recovered from dust particles deposited on acidified potato dextrose agar in Petri places

Fungal genera or species identified so far include Alternaria, Aureobasidium, Aspergillus niger, Aspergillus fumigatus, Clasdosporium, Penicillium, Rhizopus, and Stemphylium. Of these fungal microorganisms, many have been reported to be associated with medical conditions. For example, Aureobasidium, a fungus with yeast-like growth known for its allergenic properties (asthma and hay fever), may become an opportunistic pathogenic microorganism associated with health issues such as pulmonary mycosis. Similarly, other fungal microorganisms such as Aspergillus niger and Aspergillus fumigatus are also associated with medical conditions including aspergillosis.

5. Summary

We successfully started the sampling of fungal organisms in the study region at several locations. A procedure has been practiced and now has been put into practice with several student assistants taking the observations now and into FY13. It will be informative to see any differences in number and species between the low wind samples compared to the ones during the thunderstorms. Taking the opportunity to collect fungal samples during the Palomas-

Columbus PM_{10} study allowed us to collect specimens around a vacant animal feeding operation. Although the particles were not from fresh cattle manure they were likely resuspended by the wind as dry bioaerosols. During the study, three exceedances of the 24-hour PM_{10} National Ambient Air Quality Standards were observed in Palomas and two in New Mexico. We will be investigating to see if any of those samples can be cultured for fungal species. The consequences for analyzing these samples is important since these two dust plumes not only affected the Palomas/Columbus area but most of southern New Mexico and parts of southern Great Plains as the dust plumes were transported by strong southwesterly winds.

6. Next Steps and Recommendations

Clearly the most important next step is to obtain samples within a concentrated feeding operation. As this project always has been and will remain a research study, the goal is not to collect data for enforcement but to characterize the air for a taxonomic database at locations on an off the facilities.

While the past sampling techniques are passive with the particles settling on the media with natural winds, a next step is to collect size segregated or separated samples that are inhalable. We do have at our disposal three sampling instruments that can collect fungal organisms that are small, down to a few microns. The first is a multi-stage cascade impactor for use in biological aerosols. The other is a button sampler that can be worn on a person with a battery powered pump. The button sampler has been superior to the rotorod sampler in collecting small biological particle (Adhikari et al., 2003). This sampler accepts 25mm filter media that can be either prepared for fungal samples or standard mass concentrations. Third is a BioStage single-stage Viable Cascade Impactor that accepts standard Petri plates in the filter holder. This sampler's median cut-point (D_{50}) is 0.6 µm in diameter at 24 lpm which will filter out most all of the larger fungi, dust, and other aerosols, and focus on the smaller biological particles that travel deep into the lungs and are a concern for health effects

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