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ABSTRACT

Recent air analysis in the Phoenix metropolitan region has shown that particulate matter mass concentrations in urban areas are 3 to 7 times higher than nearby rural areas. This increase coincides with a dramatic increase in the number of reported incidents of Valley Fever (caused by the fungus, *Coccidioides immitis*) in Arizona and provides a rationale for investigating the microbial fraction of airborne particulate matter. The goal of this research is to develop and test methodology to quantitatively correlate the mass or number concentration of airborne fungal spores to the total mass of airborne particles. The development of these protocols and pilot data will be used to build a broad based research effort to correlate exposure of airborne biological agents with commonly monitored (and regulated) PM10 and PM2.5. Specific research objectives within this pilot investigation include the following: (i) measure the outdoor airborne fungal concentration (total and culturable) and total particle mass concentration in rural areas during seasons of high risk (June-July, October-November in Arizona), (ii) measure the same ratio at urban areas containing high particulate matter concentrations (on-going construction sites, highways, etc.) and, (iii) use rDNA gene-based technology to detect and identify the fungal spore *C. immitis* in airborne samples.

INTRODUCTION

The fungus, *Coccidioides immitis*, is only found in the soils of semi-arid regions of the southwestern United States and is the causative agent of Coccidioidomycosis or Valley Fever (Figure 1).

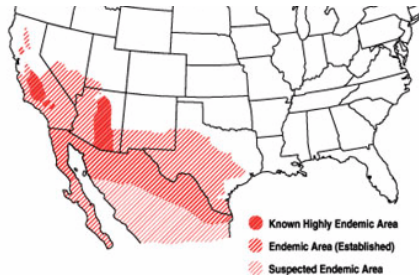


Figure 1: Distribution of Valley Fever throughout the United States and Mexico
Source: <http://www.valleyfever.com/whatis.htm>

Each year, close to 100,000 new infections arise from *C. immitis* in the U.S. The infection emanates from inhaling the spores aerosolized from disturbances in the soil (Figure 2).

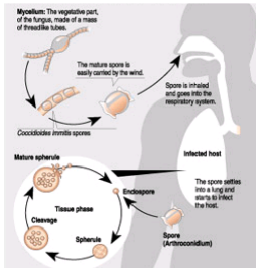
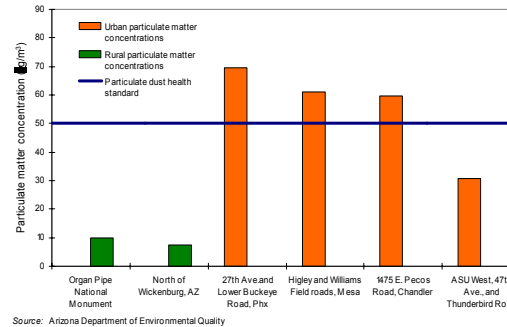


Figure 2: Life cycle of *C. immitis*
Source: <http://www.valleyfever.com/whatis.htm>

Dust storms, natural disasters, prolonged drought, and other natural environmental conditions can lead to increased incidences of Coccidioidomycosis and other airborne diseases (CDC 2000; Fisher et al. 2000; Flynn et al. 1979; Schneider et al. 1997; Stevens 1995). Human activities that disturb the soil such as road construction and traffic substantially increase particulate matter (including aerosolized spore) concentrations in urban areas. Recent studies in the Phoenix metropolitan region have shown that particulate matter concentrations in urban areas are 3 to 7 times higher than in nearby rural areas (Figure 3).



Source: Arizona Department of Environmental Quality

Figure 3: Average annual particulate matter levels in 1999 (µg/m³)

Airborne particulate matter is diverse both in size and source. Particles approximately 10 microns in diameter (PM10) or less come mainly from windblown dust while particles at 2.5 microns (PM2.5) or less are byproducts of fuel combustion. (Figure 4).

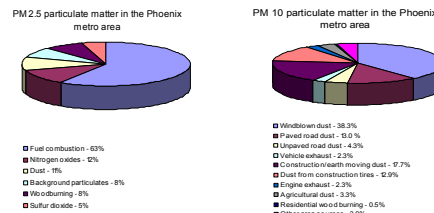


Figure 4: Distribution of PM 2.5 and PM10 sources (Arizona Department of Environmental Quality)

Airborne biological concentration data can be particularly useful in urban settings, where the particulate matter levels are significantly higher, the sources of particulate matter are diverse, and the chance of infection is exacerbated by a higher population density. The measurement of specific airborne microorganisms, however, is often difficult due to the low concentrations in the air. To this date, neither *Mycobacterium tuberculosis*, the causative organism of tuberculosis, nor *C. immitis* has ever been isolated from an airborne sample.

OBJECTIVES

The short-term objectives of this project include:

- Estimating the biological fraction of dust particles 2.5 microns and 10 microns in size in the Phoenix metro area and classifying the biological fraction as fungi or bacteria.
- Developing a correlation between PM2.5 and/or PM10 and associate fungal concentrations.

Quantifying the seasonal differences in the biological concentration of urban and rural particulate matter in the metro area.

METHODS

Developing a protocol for detecting low airborne levels of *C. immitis* through DNA amplification and sequencing methods.

Conduct outdoor sampling in:

High risk rural areas during high risk seasons (June-July, October-November) in Arizona,

Low risk rural areas,

High risk urban areas (i.e. sampling at construction sites and highways) and organisms with epi-fluorescent microscopy (Figure 5a).

Low risk urban areas (i.e. pavement and grass covered soil).
Isolate *C. immitis* from particulate matter by comparing sequences of ribosomal DNA using polymerase chain reaction (PCR) mediated amplification in gel agarose. Highly specific primers have already been developed to identify the mass of airborne biological and non-biological matter using a filter-based virtual impactor sampler.

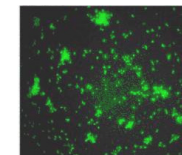


Figure 5a: Epi-fluorescent micrograph of DTAF stained airborne microorganisms.

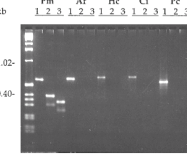


Figure 5b: Agarose gel electrophoresis for selected fungi (LoBuglio and Taylor (1995))

EXPECTED DATA

High-risk urban areas that contain the most disturbances to the soil are hypothesized to contain the highest biological fraction of the total particulate matter mass. A certain percentage of the particulate matter should contain fungal organisms. *C. immitis* is expected to comprise a very small fraction, if any at all, of the fungal loading. It is hypothesized that PM 10 will have a higher ratio of airborne fungal spore concentration to total airborne particle mass than PM 2.5, as the spores, themselves, are often larger than 2.5 microns.

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