FINAL REPORT

Assessing the Impacts of Climate and Land Use/Land Cover Change on Valley Fever Incidence

SERDP Project RC-2650

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The causative agents of coccidioidomycosis, also known as valley fever, are Coccidioides immitis and C. posadasii. The infection occurs through inhalation and cases of valley fever have been reported among military personnel in Coccidioides endemic areas of the desert Southwest since World War II, indicating an ongoing threat to military staff and families.

This limited scope project aimed to determine the prevalence of the fungus in regions that may impact military and civilian DoD personnel, if some associations between soil properties and the presence of the pathogen could be established, and if additional information about potential pathways for exposure could be identified.

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ACRONYMS AND ABBREVIATIONS

AFB	Air Force Base
BSC	Biological Soil Crusts
CDC	Center for Disease Control
CDPR	California Department of Pesticide Regulation
CSUB	California State University, Bakersfield
DGGE	Denaturing Gradient Gel Electrophoresis
DRI	Desert Research Institute
EAF	Environmental Analysis Facility
EAFB	Edwards Air Force Base
LS	Limited Scope [SERDP] project
MCAGCC	Marine Corps Air Ground Combat Center
NAS	Naval Air Station
NNDSS	National Notifiable Diseases Surveillance System
PCR	Polymerase Chain Reaction
PI-SWERL®	Wind tunnel-like device to measure and collect wind suspendable dust from surfaces
PM10	Particulate matter with aerodynamic diameter smaller than 10 micrometers.
TRAKER™	Vehicle based system to measure and collect vehicle-suspendable road dust
USDA	US Department of Agriculture

KEYWORDS

Fungus, Pathogen, Dust, Mojave Desert, Coccidiodes, PM10, Road Dust, Mineral Dust, Health Effects

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ABSTRACT

OBJECTIVES

The causative agents of coccidioidomycosis, also known as valley fever, are *Coccidioides immitis* and *C. posadasii*, two species of ascomycete fungi in the order of the Onygenales. Although it is known that these fungi grow in the soil of endemic areas, that military personnel are impacted by the disease, and that infection occurs through inhalation, relatively little is known about the specific conditions under which exposure occurs. Cases of valley fever have been reported among military personnel in *Coccidioides* endemic areas of the desert Southwest since World War II, indicating an ongoing threat to military staff and families.

This limited scope project aimed to determine the prevalence of the fungus in regions that may impact military and civilian DoD personnel, if some associations between soil properties and the presence of the pathogen could be established, and if additional information about potential pathways for exposure could be identified. The specific objectives were to determine/identify:

- any measurable soil-, or environmental variables or combinations thereof that are promising as proxies for the presence of live *Coccidioides* spp. in soils.
- if potential for wind erosion and dust emission can be related to exposure to the pathogen.
- if dust suspended from travel on unpaved roads is related to exposure to the pathogen.
- if there are promising parameters that can be linked to large-scale datasets, maps, and satellite retrievals for the purpose of scaling field results up to the installation- or regional-scale.

TECHNICAL APPROACH

Methods employed to accomplish the stated objective included a combination of bulk soil sampling (5-7 cm depth in general, and several core samples down to 30 cm), collection of wind suspendable dust from soil surfaces that tested positive for the presence of *Coccidioides* using a portable wind tunnel-like device (PI-SWERL®), and collection of road dust suspended by the travel of a vehicle on unpaved roads using an established mobile platform (TRAKER™). Bulk soil sampling occurred three times over the course of the project year to capture temporal variations in the vicinity of three DoD installations in endemic areas of the southwestern US. Those were the Naval Air Station (NAS) at Lemoore, Edwards Air Force Base (EAFB), and the Marine Corps Air Ground Combat Center (MCAGCC) at Twentynine Palms including a few locations from nearby Joshua Tree National Park. After the first collection period in winter, sites that tested positive for *Coccidioides* were targeted for core sample collection, wind-suspendable dust collection, and suspended road dust collection during the ensuing spring and summer sampling campaigns.

Samples collected during field work were subjected to standard analysis for selected physical and chemical parameters such as grain size, pH, electrical conductivity, and total dissolved salts, as well as inorganic ions. All samples were analyzed for the presence of the pathogen using DNA extraction and nested polymerase chain reaction (PCR) methods. Two different diagnostic primer pairs that were rather specific to *Coccidioides* spp. were used to test for the presence of the pathogen.

Additionally, fingerprints of the broader fungal community in the samples were obtained using standard environmental microbiological techniques. Although RNA extraction was embarked upon for the purpose of identifying pathogen active growth sites, that effort was resource intensive and had to be postponed for a possible future research project.

RESULTS

Coccidioides spp. were detected in soil and dust samples collected near all three military installations, with most of the positive soil samples associated with the wet season (end of January – beginning of February). The Twentynine Palms area showed the highest percentage of *Coccidioides* positive samples. Soil core samples collected at *Coccidioides* positive sites revealed the presence of the pathogen predominantly, but not exclusively near the soil surface (A and B horizon). Soil fungal communities were composed of few species that varied with soil depth.

The sample analyses indicated that elevated pH (7.1 – 8.1), electrical conductivity, total dissolved salts, and silt and clay content can be helpful in describing a suitable *Coccidioides* habitat, but none of these appeared to be first order parameters in controlling the occurrence of the fungus. There is inferential evidence that microbial antagonists to the pathogen and the type of organic matter in the soil may have an influence. Of interest were also our results of soil ion chromatography, indicating high amounts of soluble calcium and potassium in desert soils where *Coccidioides* seemed to be more prevalent. The range of soil parameters measured provide an envelope of necessary conditions for the growth of *Coccidioides*, but do not prescribe the conditions that are sufficient for the fungus to thrive. One important finding is that the fungus is nearly absent from agricultural fields with high levels of sulfate and nitrate, suggesting that once lands have been severely disturbed from their original state, resultant changes in nutrients and a shift in soil microbial diversity becomes unsuitable for fungal growth.

The pathogen was detected via diagnostic PCR in several wind-suspendable dust samples that were collected with the PI-SWERL method. However, most of the sites that tested positive for the presence of *Coccidioides* with a bulk surface sample tested negative for material that was resuspended from the surface using the PI-SWERL. This suggests that soils where the fungus is relatively abundant are also soils that are less prone to erosion. With regard to suspended road dust, the limited number of samples collected does not allow for in-depth understanding of the relationship between road dust and exposure to *Coccidioides*. However, road dust from the Twentynine Palms area did test positive for the fungus, confirming that simply traveling on unpaved roads in endemic areas is a potential pathway for exposure, possibly a very important one given the extensive exposure to road dust that troops are subjected to during training activities.

One outcome of this study was the observation that *Coccidioides* growth sites appear to be restricted to where vegetation such as creosote and salt bush are still in place. Possibly, soils from these sites where rodents are frequently observed contain animal derived organic matter and the fungus prefers to catabolize rodent keratin instead of plant detritus. When the landscape is reworked to the point of becoming an agricultural field, the soil is of course, more prone to erosion and apparently no longer hospitable to *Coccidioides* growth, perhaps because of changes in soil chemical parameters and microbiota. This implies that exposure to the fungus can happen in the immediate vicinity of land disturbance activity at the time of the activity, for a finite amount of time following disturbance due to erosional events, or within a small buffer zone along the border between a vegetated growth site and an area that is prone to erosion by wind or vehicular traffic.

BENEFITS

This conceptual framework, if shown to be true, can result in tools that are immediately useful for minimizing exposure to the fungus. Satellite imagery can be used to identify large highly degraded locations or ongoing and planned construction within the setting of endemic areas of the pathogen that together with wind data indicate high risk areas for *Coccidioides* exposure. Maps can be retrieved for past years as well, which allows the documentation of changes in risk of exposure to the pathogen due to changes in environmental conditions including human activities. Together with more information about the lifecycle of the fungus, these tools can be used to inform management decisions about certain activities to reduce exposure and ultimately disease burden.

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1.0 OBJECTIVE

1.1 TECHNICAL OBJECTIVES

The causative agents of coccidioidomycosis, also known as valley fever, are *Coccidioides immitis* and *C. posadasii*, two species of ascomycete fungi in the order of the Onygenales. Although it is known that the fungus grows in the soil of endemic areas, that military personnel are impacted by the disease, and that infection occurs through inhalation, relatively little is known about the specific conditions under which exposure occurs. Broadly, this LS project aimed to determine the prevalence of the fungus in regions that may impact military and civilian DoD personnel, if some associations between soil properties and the presence of the fungus could be established, and if additional information about potential pathways for exposure could be identified. The intent was to use the information gained to determine if pursuing a larger, more in-depth investigation might be warranted on the basis that such an effort would yield actionable information that the DoD can use to reduce exposure, understand risks, and improve land management practices as appropriate.

The targeted objectives of the LS project were:

I. **Determine if there are any measurable soil-, or environmental variables or combinations thereof that are promising as proxies for the presence of live *Coccidioides* spp. in soils.** If not, then it would be difficult to identify specific conditions and locations where the fungus might be present or concentrated relative to elsewhere within the endemic regions.

II. **Determine if the susceptibility of a soil where *Coccidioides* spp. are present to wind erosion and dust emission can be related to the amount or existence of suspended arthroconidia.** If wind erosion can be shown to be a likely pathway for exposure, then it would be possible to identify conditions under which exposure is more likely.

III. **Determine if exposure to dust suspended from travel on unpaved roads is related to exposure to arthroconidia.** If so, then this has implications for military personnel who train or work in areas where the fungus is endemic even in the absence of exposure from wind erosion events.

IV. **Identify promising parameters that can be linked to large-scale datasets, maps, and satellite retrievals for the purpose of scaling field results up to the installation- or regional-scale.** Ultimately, it will not be possible to make ground-based measurements in every part of every DoD facility and nearby communities. Some means to identify areas with heightened risk needs to be developed that relies on available spatial datasets.

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2.0 BACKGROUND

2.1 COCCIDIOIDES SPP. AND COCCIDIOIDOMYCOSIS

Coccidioides spp. are obligate aerobic dimorphic fungi that form vegetative hyphae (mycelium) when growing as saprophytes in the top horizons of moist soil. These opportunistic pathogens are somehow adapted to a variety of environments that could be characterized as the Lower Sonoran Lifezone (Daubenmire 1938) that comprises arid and semi-arid areas in the southwestern U.S. and habitats with similar climate conditions and soils in Mexico and South America (Maddy and Coccozza 1964; Maddy 1965; Kolivras et al. 2001; Fisher et al. 2007; Saubolle et al. 2007; Baptista-Rosas et al. 2007 and 2012; Ampel 2010) (Figure 1). It appears that the pathogen is most successful in soils where few other microbial species compete for resources, as it has been termed a poor competitor which benefits from reduced antagonism by other microbes in alkaline-saline soils (Baptista Rosas et al. 2007). During the dry season *Coccidioides* spp. survive in form of arthroconidia which form when the fungal hyphae stop elongating and cells undergo autolysis, lose their internal contents, and form spore-like structures which are barrel-shaped, and which can easily be disrupted and become airborne. These arthroconidia are approximately 3-5 μm in length, which is small enough to remain suspended in the air for hours to days and to be inhaled into the lungs, where an infection may result.

Figure 1. Map of Coccidioides spp. Endemic Areas and Suspected Endemic Areas in the Southwestern U.S. and Mexico Based on Incidence of Coccidioidomycosis and Skin Testing.

Areas of highest incidence (highly endemic) are shaded in darker red (map based on Ochoa et al. 1967, Kolivras, 2001, and Hector and Laniado-Laborin, 2005).

Approximately half of all people that become infected with *Coccidioides* spp. show no symptoms, whereas the other half may exhibit flu-like symptoms, lasting up to several weeks.

The incidence statistics are based on confirmed diagnosed cases, which likely represent a very small fraction of all cases. Because of the non-specific symptoms of coccidioidomycosis it is believed to be profoundly underdiagnosed and its impact on lost productivity grossly underappreciated (Thompson et al. 2014). For a small percentage of those who become infected, the disease can pose a very serious health threat and may result in death. To characterize long-term national trends, the Center of Disease Control and Prevention (CDC) regularly analyzes data from the National Notifiable Diseases Surveillance System (NNDSS). A recently released report for the period between 1998 and 2011 indicated that the incidence of reported annual coccidioidomycosis increased substantially during this period, from 5.3 per 100,000 population in the endemic area (Arizona, California, Nevada, New Mexico, and Utah) in 1998 to 42.6 per 100,000 in 2011. An increase of more than 800% was reported in highly endemic areas in Arizona, California, New Mexico, and Western Texas (Figure 2).

The CDC report also pointed out that a 2006 study found only 2 to 13 percent of patients with signs and symptoms were tested for valley fever, providing additional evidence that the disease is likely greatly underreported (Chang et al. 2008; NNDSS report 2011; Brown et al., 2013). Actual and historical data on coccidioidomycosis reported incidence can be obtained from the following website: https://wonder.cdc.gov/nndss/static/2018/09/2018-09-table2C.html. It should be noted that by week 9 in 2018, reported cases of coccidioidomycosis were 21% higher compared to the same time period in the previous year (2,246 cases in 2018 vs. 1,853 cases in 2017, cumulative for the entire U.S.).

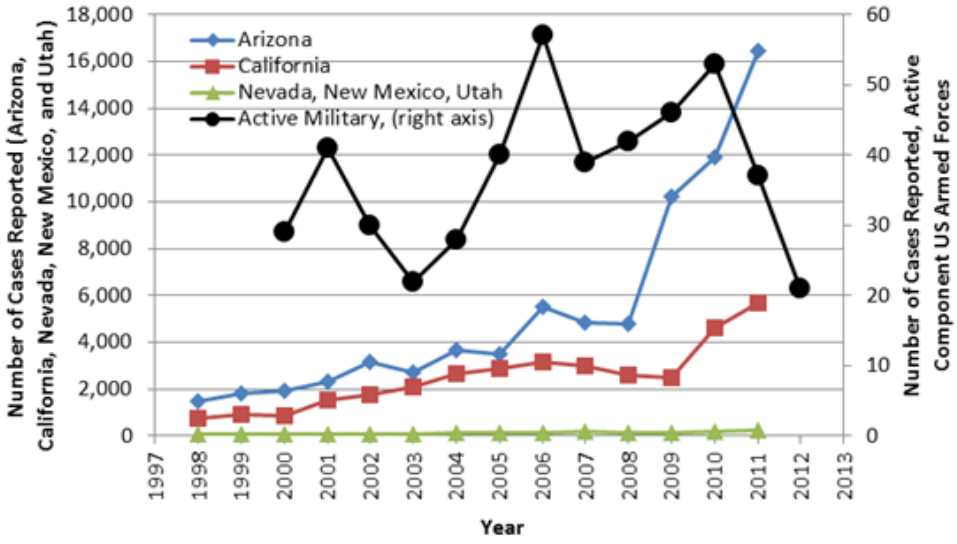


Figure 2. Coccidioidomycosis Reported Cases for Arizona, Nevada, California, New Mexico, and Utah (Centers for Disease control, 2013) As Well As for Active Component US Armed Forces 2000-2013 (Right axis) (AFHSC, 2012).

Changes in reporting protocols may have influenced apparent sharp increases in 2009 in Arizona and 2010 in California (CDC, 2013). After the peak in disease incidence in 2011, reported cases of coccidioidomycosis declined until 2014 (AZ: 5,630 cases, CA: 2,247 cases) but started to increase again according to data that are available through 2016 (AZ: 6,162 cases, CA: 3,911 cases, see https://wonder.cdc.gov/nndss/nndss_weekly_tables_2016_38.asp?mmwr_year=2016&mmwr_week=52&mmwr_table=2B&request=Submit.)

Incidence in active component military over the same period did not demonstrate a discernible, consistently increasing trend, but this may be due to noise in the data due to the sporadic nature of outbreaks and the relatively small absolute number of cases (Figure 3) in comparison to the general public in endemic areas. Moreover, unlike settings for the general population, military facilities are prone to significant fluctuations in terms of numbers and timing of occupancy, potentially resulting in fluctuations in the number of person-years spent in endemic areas. Among U.S. service members, approximately half of the incident cases were diagnosed in California and a fifth in Arizona (The Armed Forces Health Surveillance Center [AFHSC], 2010 and 2014). In these reports, incidence rates appear to have been highest at the Lemoore NAS, Edwards AFB, China Lake NAWS, and the Fort Irwin NTC, all in California, as well as at Davis-Monthan AFB near Tucson and Luke AFB near Phoenix, both in Arizona. In terms of the absolute number of cases, among 511 incidents (2000-2013), there were 68 at Lemoore, 63 at Davis-Monthan, and 53 at the Naval Station in San Diego (MAGCC Twentynine Palms was not mentioned). Further back in time, cases of valley fever have been frequently reported among military personnel training in *Coccidioides* endemic areas since World War II (e.g. Drips and Smith 1964; Hooper et al. 1980; Standaert et al. 1995; Gray et al. 1998; Olivere et al. 1999; Crum et al. 2002, 2003 and 2004) indicating an ongoing threat to military staff and the general public in the vicinity of those military bases affected by the pathogen (see also figure 4).

It should also be noted, that an abrupt increase in the rates of coccidioidomycosis has been documented for construction workers and farm laborers (Das et al. 2012), children (McCarty et al. 2013), and incarcerated populations (Pappagianis 2007; Burwell et al. 2009) which has resulted in higher rates of hospitalizations for coccidioidomycosis (Sondermeyer et al. 2013).

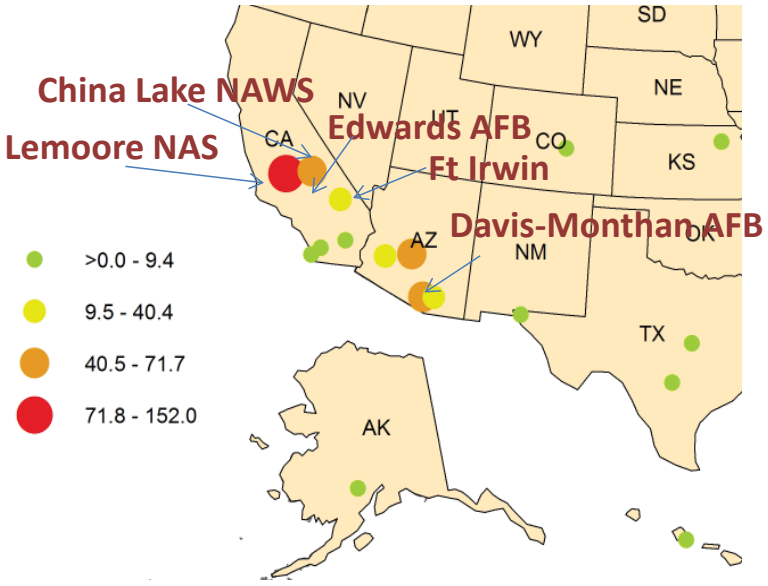


Figure 3. Incident of Coccidioidomycosis Within the Active Component, U.S. Armed Forces, 2000-2012 per 100,000 Persons/Year (Adopted from AFHSC, 2012, 19:10, Brief Report).

Development of a vaccine has been elusive to date, and accurate diagnosis and treatment of the disease can be difficult (Thompson et al. 2014). Therefore, prevention through reasonable reduction of exposure is likely the best way to reduce incidence of the disease and the associated human and financial losses.

Although the causal relationships are not well known at this time, it is expected that exposure to arthroconidia of *Coccidioides* spp. will be influenced by factors that impact the organisms' life cycle as well as human exposure to airborne dust sources. The ongoing drought in the southwestern U.S. has also contributed to increased dust development in many areas, and a drier climate is predicted for this area in the near future (Dettinger et al. 2012). Excessive ground-water use and saline intrusion to the aquifer has led to an increase in abandoned agricultural fields in some areas of the southwestern U.S. and Mexico including the Mojave and Sonoran Deserts (e.g. Castellanos et al. 2005; Abella et al. 2010).

The larger vegetation in the Mojave Desert is generally scattered due to the limited amount of water available. But the area between creosote bushes (*Larrea tridentata*) and salt bushes (*Atriplex* spp.) is filled with biological activity. Biological soil crusts (BSC) cover large areas between shrubs and are not only important for the nutritional status of desert soils, but BSCs also enhance soil quality by aggregating soil particles, thereby reducing wind and water erosion (Mazor et al. 1996; Belnap and Eldridge 2003). However, extensive disturbance poses a risk of covering or destroying biological soil crusts, preventing photosynthesis, and ultimately causing their degeneration and death. Biological soil crusts need many years to develop and once destroyed can be difficult to rehabilitate, leaving bare soil behind, vulnerable for further erosion (Belnap and Gillette 1998; Bowker 2007). Remote sensing of biological soil crusts in the Mojave Desert revealed the loss of cyanobacterial crusts and lichens (Ustin et al. 2009).

The impacts of military vehicle traffic on natural areas were addressed by Anderson et al. (2005 and references within) and concerns were raised about off-road recreational activities in general in semi-arid areas of the southwestern US. Recovery attempts of soils and vegetation in military base and training camps were conducted after World War II in some areas of the Mojave and Sonoran Deserts (Prose and Mezger 1985; Kade and Warren 2002). These studies indicate that recovery of compromised desert soils and vegetation to pre-disturbance status takes at least several decades, sometimes more than a century.

Characteristics of individuals with fungal cause of death listed for the period 1970-2012 (N=216) in the US Air Force Mortality Registry.

Variable	n	%N
Gender		
Male	211	97.69
Female	4	1.85
Unknown	1	0.46
Age Group		
20-44 years	18	8.33
45-54 years	16	7.41
55-69 years	50	23.15
65 years and older	135	61.11
Race		
White	167	77.31
Black	32	14.81
Hispanic	4	1.85
Unknown	13	6.02
Underlying Cause of Death		
Aspergillosis	36	16.67
Blastomycosis	4	1.85
Candida	43	19.91
Coccidioidomycosis	32	14.81
Cryptococcosis	12	5.56
Histoplasmosis	12	5.56
Mucormycosis/Zygomycosis	7	3.24
Pneumocystosis	25	11.57
Sporotrichosis	2	0.93
Unspecified mycosis	43	19.91
Duty Status		
Active Duty/Active Reserve	8	3.70
Retiree	208	96.29

Figure 4. Mortality from Fungal Disease in the US Airforce 1970-2013 (Reeves and Kugblenu. 2016).

Note the large number of unspecified mycoses. It is not unlikely that some of these were caused by Coccidioides spp.

Previously performed research in 2014 at several sites at Edwards AFB indicated that individual soil parameters, such as pH and grain size alone, are not reliable indicators of a growth site of the pathogen. Furthermore, the fact that dormant arthroconidia of the pathogen can withstand hostile environmental conditions for an unknown amount of time (e.g. non-disturbed *C. immitis* positive site transformed into an agricultural field) makes it impossible to distinguish 'active sites' from 'dormant sites' or 'accumulation sites' (see Fisher et al. 2007 for definitions). These sites can be called 'hot spots' of the pathogen and are presumed to be the most relevant regarding airborne arthroconidia when disturbed The use of DGGE fingerprinting in microbial community analyses is helpful in determining the dominant members of microbial communities, which will appear as strong distinct bands in the DGGE profiles (Green et al. 2010).

It has been hypothesized that exposure to the fungal arthroconidia is related to the emission of dust from arid soils where the pathogen is endemic, due to disturbance by humans or due to natural causes.

However, this link has not been established with direct evidence. Furthermore, it is not clear if exposure is a result of regional high winds that suspend the arthroconidia from large, diffuse source regions within the endemic areas (which can encompass numerous "hot spots") or if there are a limited number of much smaller source areas that are the primary culprits for infection.

2.2 HYPOTHESES AND PROOF OF CONCEPTS

Hypothesis 1: The growth of the pathogen *Coccidioides* is supported in soils that are characterized by an elevated pH (between pH 8 and 9) and high concentrations of silt and clay (at least 60%) and possibly other parameters. If true, this would provide a basis for using large scale spatial data to narrow growth areas for *Coccidioides*.

Approach:

Soils around 3 military bases in endemic areas of the pathogen were collected based on a sampling plan that focused on soils with different chemical and physical parameters. The presence of the pathogen was investigated using a culture independent molecular biological approach. Additional information about the vertical distribution of the fungus was sought at several locations. Understanding the depth profile for *Coccidioides* growth may be critical for understanding exposure routes.

Hypothesis 2: The exposure to arthroconidia of the pathogen is elevated in dry times of the year with increased wind activity that follows favorable growth conditions for *C. immitis*. If true, this would provide the missing link between where the fungus is found and how humans are exposed to it. In turn, this information can be used to model exposure and manage risks.

Approach:

Wind-suspendable dust samples were collected with a portable wind tunnel (PI-SWERL) at the end of summer, 2017, when soil conditions were dry at sites that were known to be positive sites for *C. immitis* based on soil sampling performed in the prior winter and spring. Soil cores were collected and sampled at 5 cm depth intervals down to 30 cm. The premise for this is that if *Coccidioides* are present in deeper soil layers, then activities beyond surficial wind erosion, such as trench digging, or construction could cause arthroconidia to become airborne and cause infection in addition to disturbed soil surfaces.

Hypothesis 3: Suspended dust from travel on unpaved roads is a significant exposure pathway. If true, this would suggest that even traveling through endemic areas on unpaved roads could be sufficient to result in exposure. This has implications for training scheduling decisions, especially for *C. immitis*-naïve personnel, who may be cycling through a particular facility as part of routine training.

Approach:

Dust samples were collected via the TRAKER method from unpaved roads at the end of summer, 2017 adjacent to sites that were known to be positive sites for *C. immitis* based on soil sampling performed in the prior winter and spring.

3.0 MATERIALS AND METHODS

3.1 SITE SELECTION

Soil samples were collected from surface soils around three military facilities located in valley fever endemic areas with prior history of disease incidences among personnel. Those were Naval Air Station (NAS) Lemoore, Edwards Airforce Base (EAFB), and Twentynine Palms Marine Corps Air Ground Combat Center (MCAGCC). Although, at the proposal stage there was interest from all three facilities to provide logistical support for conducting measurements within the facility boundaries, it was not possible to secure final permission for that purpose within the time constraints of this pilot project. Therefore, efforts were targeted at collecting samples from publicly accessible locations around the facilities. Information available from the USDA Web Soil Survey database revealed that each individual location has diverse soil types and differs in soil chemical and physical parameters. By sampling along informal transects, we were able to obtain samples from different soil types near each location (table 1).

NAS Lemoore, Lemoore, CA: This site is situated in the Central Valley of California which is characterized by intense agriculture and comparatively high incidence of coccidioidomycosis (figure 3). NAS has ~20,000 active military, civilians and dependents and obtains about 6.4 inches of rainfall annually (Figure 5).

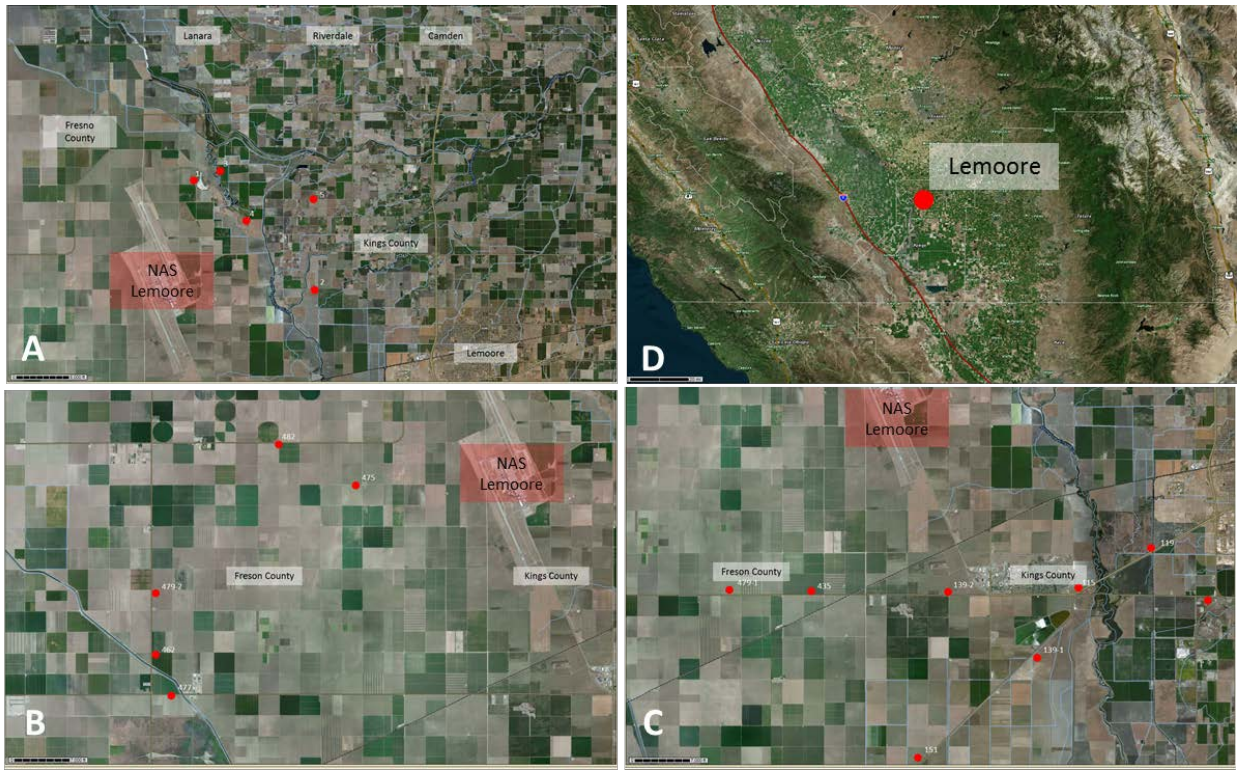


Figure 5. Sampling Spots Near NAS Lemoore (indicated as red dots).

A: *west of the station,* ***B:*** *east of the station,* ***C:*** *south of the station,* ***D:*** *overview of location in the central valley of California.*

Table 1. Coordinates of Sampling Sites Near NAS Lemoore with Indication of Soil Type and Soil Map Unit Number as Obtained from the USDA Websoilsurvey Database.

Location	Soil Type and Map Unit	Site Description	Coordinates	
LM-1 (N26)	Panoche clay loam, saline-alkali (151)	dirt road between agricultural fields and eucalyptus forest towards a dry, salty lakebed	36.37749 N	119.93245 W
LM-2	Vanguard sandy loam, partially drained (168)	agricultural field	36.32841 N	119.86842 W
LM-3	(no data) water, either Vanguard sandy loam, partially drained (168), or Gepford clay, partially drained (115)	area with ponds near creek, salty soils, plenty of salt bushes (*Atriplex* spp.), high animal activity (birds and mammals)	36.37970 N	119.92158 W
LM-4	Gepford clay, partially drained (115)	dirt road along small canal, close to new orchard	36.35488 N	119.90508 W
LM-5	Grangeville fine sandy loam, saline-alkali, partially drained (121)	orchard with young almond trees	36.36556 N	119.87218 W
LM-482	Calflax clay loam, saline-sodic, wet, 0-1% slope (482)	agricultural field	36.34256 N	120.04868 W
LM-475	Posochaet clay loam, saline-sodic, wet 0-2% slopes (479)	agricultural field	36.32813 N	120.01418 W
LM-479-1	Cerini clay loam, 0-2% slope (479)	agricultural field	36.25557 N	120.01647 W
LM479-2	Cerini clay loam, 0-2% slope (479)	agricultural field	36.29001 N	120.10330 W
LM-462	Ciervo, wet Ciervo complex, saline-sodic, 0-1% slope (462)	agricultural field, not in use meadow with plenty of ladybugs	36.26982 N	120.10332 W
LM-477	Westhaven clay loam, 0-2% slope (477)	almond orchard	36.25518 N	120.08430 W
LM-435	Lethenet clay loam, 0-2 % slope (139)	agricultural field	36.25545 N	119.98242 W
LM139-1	Lethenet clay loam (139)	Unused agricultural field, grasses, clay rich area	36.23450 N	119.88711 W
LM139-2	Lethenet clay loam (139)	abandoned agricultural field, high grasses and shrubs	36.25569 N	119.92308 W
LM-115	Gepford clay, partially drained (115)	unused agricultural fields with grass	36.25727 N	119.92308 W
LM-151		agricultural field	36.19823 N	119.93445 W
LM-119	Grangeville sandy loam, saline-alkali (119)	along a dirt road between agricultural fields towards a meadow, plenty of birds	36.27182 N	119.83556 W
LM-J	Lemoore sandy loam, partially drained (137) Boggs sandy loam, partially drained (103)	salty environment with Iodine bushes (*Allenrolfea occidentalis*), was flooded in winter	36.25462 N	119.81111 W

Edwards AFB, Rosamond, CA: The base is located on the Western edge of the Mojave Desert of California. Near the base are several dry lake beds surrounded by desert shrubs. The surrounding land has been converted to ranch land, agricultural fields, but still contains areas with natural desert shrub. The Western Mojave Desert is undergoing intense renewable energy, and especially solar power development. Edwards AFB has ~18,000 active military, civilians and dependents. The area receives about 7.4 inches of rainfall annually. Incidence of coccidioidomycosis is high (figure 3). Prior research in this area by our group has confirmed the presence of the pathogen in soil and dust. See Figure 6 and table 2 for detailed information about the location of all sampling spots, as well as information about soil types and vegetation cover.

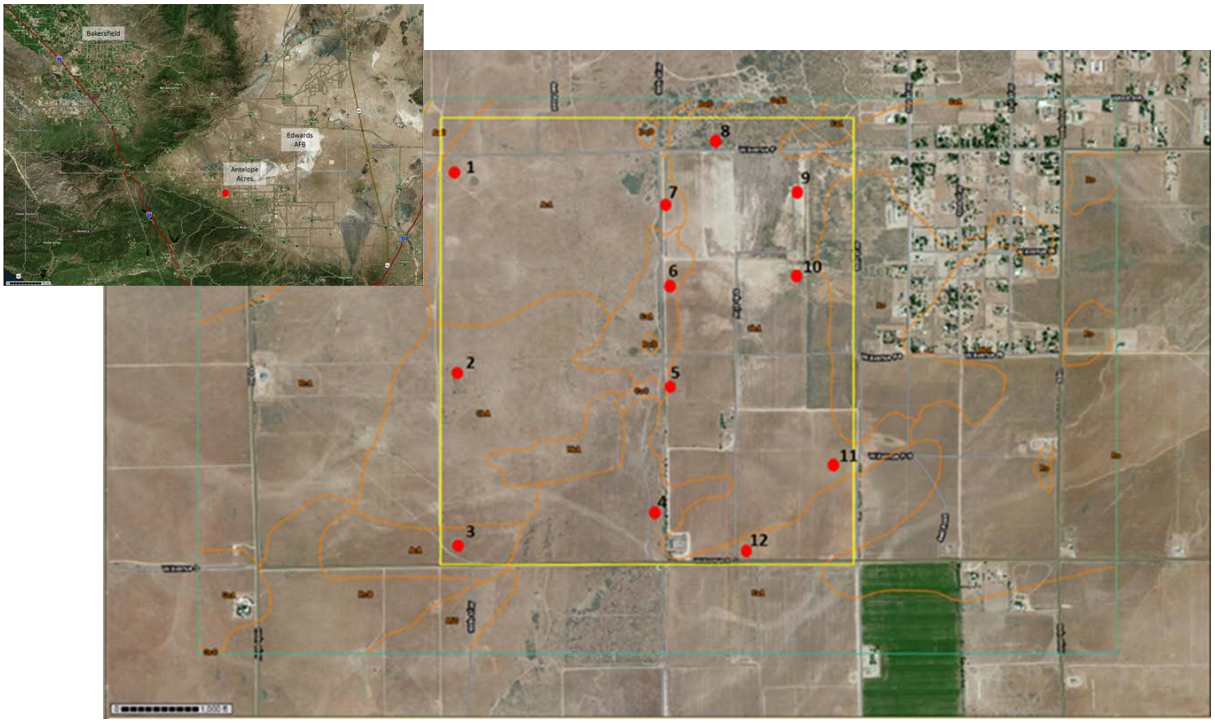


Figure 6. Sampling Spots West of Edwards Airforce Base and West of Antelope Acres (indicated as red dots).

The smaller photo in the upper left corner shows the location of EAFB in the Southwestern Mojave Desert and also shows the sampling site (red dot).

Table 2. Coordinates of Sampling West of Edwards Airforce Base and West of Antelope Acres with Indication of Soil Type, Percent Slope, and Soil Map Unit Number, as Obtained from the USDA Websoilsurvey Database.

Location	Soil Type and Soil Map Unit	Site Description	Coordinates	
AA-1	Adelanto coarse sandy loam, 2 to 5 percent slopes (AcA)	grassland, mostly invasive *Bromus* spp. and native and non-native annuals	34° 44’ 48.97” N	118° 18’ 57.87” W
AA-2	Cajon loamy sand, loamy substratum, 0 to 2 percent slopes (CbA)	grassland, mostly invasive *Bromus* spp. and native and non-native annuals	34° 44’ 23.01” N	118° 18’ 59.69” W
AA-3	Cajon loamy sand, loamy substratum, 0 to 2 percent slopes (CbA)	grassland, mostly invasive *Bromus* spp. and native and non-native annuals	34° 43’ 59.80” N	118° 18’ 59.03” W
AA-4	Cajon loamy sand, 0 to 2 percent slopes (CaA)	eroded landscape with scattered vegetation, mostly native and non-native annuals	34° 44’ 04.22” N	118° 18’ 25.29” W
AA-5	Cajon loamy sand, loamy substratum, 0 to 2 percent slopes (CbA)	eroded landscape with scattered vegetation, mostly native and non-native annuals	34° 44’ 20.40” N	118° 18’ 24.36” W
AA-6	Cajon loamy sand, 0 to 2 percent slopes (CaA)	eroded landscape with scattered vegetation, mostly native and non-native annuals	34° 44’ 34.60” N	118° 18’ 24.82” W
AA-7	Adelanto coarse sandy loam, 2 to 5 percent slopes (AcA)	eroded area dominated by rabbit brush (*Ericameria nauseosa*)	34° 44’ 42.86” N	118° 18’ 25.24” W
AA-8	Cajon loamy sand, loamy substratum, 0 to 2 percent slopes (CbA)	eroded area dominated by rabbit brush (*Ericameria nauseosa*)	34° 44’ 50.91” N	118° 18’ 16.91” W
AA-9	Cajon loamy sand, loamy substratum, 0 to 2 percent slopes (CbA)	eroded landscape with scattered vegetation, mostly native and non-native annuals	34° 44’ 48.56” N	118° 18’ 03.94” W
AA-10	Cajon loamy sand, loamy substratum, 0 to 2 percent slopes (CbA)	eroded landscape with scattered vegetation including tumble weeds (*Salsola* sp.)	34° 44’ 35.76” N	118° 18’ 03.89” W
AA-11	Cajon loamy sand, loamy substratum, 0 to 2 percent slopes (CbA)	abandoned agricultural field with scattered growth of invasive grasses (*Bromus* spp. and others)	34° 44’ 11.53” N	118° 17’ 55.99” W
AA-12	Cajon loamy sand, 0 to 2 percent slopes (CaA)	grassland, mostly invasive *Bromus* spp. and native and non-native annuals	34° 43’ 58.90” N	118° 18’ 10.16” W

Twentynine Palms MCAGCC: The Marine Corps Air to Ground Combat Center is situated in the Southern Mojave Desert. It hosts ~50,000 active military, civilians and dependents. Incidence of coccidioidomycosis is comparatively low (figure 3). The area is characterized by scattered desert shrub and receives about 4.4 inches of rainfall annually (figure 7, table 3). No soil data were available from the USDA websoil survey database for this area. However, data were available for sampling sites in Joshua Tree NP. These soils were characterized as Morongo, Pinecity and Nasagold loamy sands (landform: fan aprons), sometimes gravelly, embedded in Jumborock outcrop associations (figure 7, table 3).

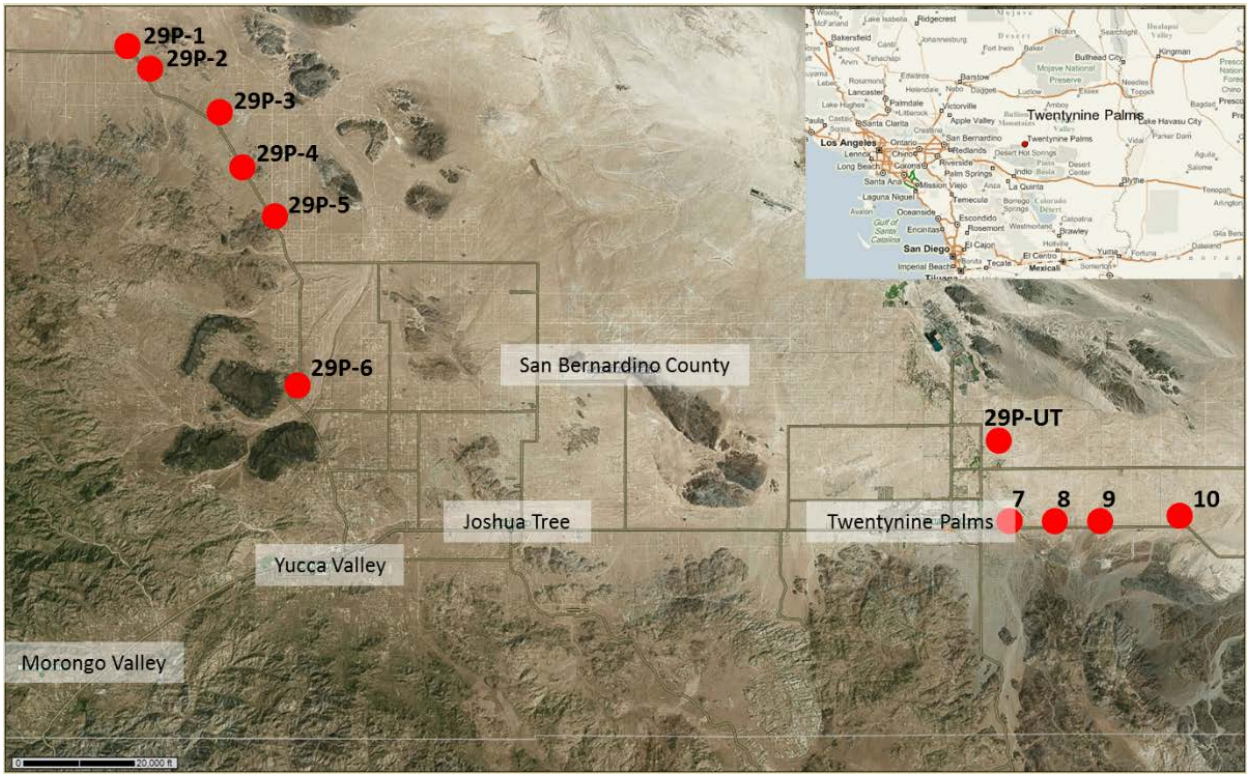


Figure 7. Sampling Spots Near Twentynine Palms MCAGCC (indicated as red dots).

The location of Twentynine Palms is indicated in map in the upper-right corner of this figure.

Table 3. Coordinates of Sampling Sites Near Twentynine Palms MCAGCC.

Information about soil type and soil map unit was not available from the USDA websoilsurvey database.

Location	Soil Type and Soil Map Unit	Site Description	Coordinates	
29P-1	no digital data available in USDA websoilsurvey database	landscape with scattered creosote bushes (*Larrea tridentata*) and salt bushes (*Atriplex* spp.)	34° 22' 08.46" N	116° 32' 24.39" W
29P-2	no digital data available in USDA websoilsurvey database	landscape with scattered creosote bushes (*Larrea tridentata)* and salt bushes (*Atriplex* spp.)	34° 21' 40.37" N	116° 31' 55.75" W
29P-3	no digital data available in USDA websoilsurvey database	area with large boulders, graffiti, trashed	34° 20' 05.00" N	116° 29'18.79" W
29P-4	no digital data available in USDA websoilsurvey database	landscape with scattered creosote bushes (*Larrea tridentata*) and salt bushes (*Atriplex* spp.) including occasional Joshua trees *(Yucca brevifolia*)	34° 18' 37.24" N	116° 28' 15.72" W
29P-5	no digital data available in USDA websoilsurvey database	landscape with scattered creosote bushes (*Larrea tridentata*) and salt bushes (*Atriplex* spp.) including occasional Joshua trees *(Yucca brevifolia*), very rocky	34° 17' 10.79" N	116° 27' 13.62" W
29P-6	no digital data available in USDA websoilsurvey database	landscape with scattered creosote bushes (*Larrea tridentata*) and salt bushes (*Atriplex* spp.) including occasional Joshua trees *(Yucca brevifolia*), eroded soil	34° 15' 35.74" N	116° 26' 23.01" W
29P-7	no digital data available in USDA websoilsurvey database	landscape with scattered creosote bushes (*Larrea tridentata*) and salt bushes (*Atriplex* spp.), lots of ants	34° 08' 11.17" N	116° 01' 04.85" W
29P-8	no digital data available in USDA websoilsurvey database	landscape with scattered creosote bushes (*Larrea tridentata*) and salt bushes (*Atriplex* spp.), eroded soil, rodent activity high	34° 08' 11.85" N	115° 00' 09.16" W
29P-9	no digital data available in USDA websoilsurvey database	landscape with scattered creosote bushes (*Larrea tridentata*) and salt bushes (*Atriplex* spp.), some beavertail cacti (*Opuntia basilaris*)	34° 08' 13.90" N	115° 58' 10.91" W
29P-10	no digital data available in USDA websoilsurvey database	landscape with scattered creosote bushes (*Larrea tridentata*) and scattered salt bushes (*Atriplex* spp.), lots of ants	34° 08' 14.75" N	115° 54' 50.02" W
UT	no digital data available in USDA websoilsurvey database	eroded landscape, scattered saltbushes, mostly unvegetated	34° 11' 41.84" N	116° 02' 06.72" W
Joshua Tree National Park				
SR	Desertqueen-Jumborox-Rock outcrop association, 2 to 8 percent slopes, warm	Joshua Tree NP, Skull Rock trail, boulders, lots of organic matter, desert oaks (*Quercus* sp.) and many other shrubs	33° 59' 50.28" N	116° 03' 37.44" W
HH	Morongo loamy sand, 2 to 4 percent slopes	Joshua Tree NP, Hall of Horrors Trail, scattered Joshua trees, salt bushes (*Atriplex* spp.) and other bushes	34° 00' 02.16" N	116° 08' 45.96" W
HV	Rock outcrop	Joshua Tree NP, Hiddden Valley, lots of organic matter, desert oaks, shrubs and yucca plants	34° 00' 12.00'N	116° 10' 25.32" W

3.2 SOIL SAMPLING

The collection of soil samples focused on identifying conditions that are likely to encourage the presence of *Coccidioides* spp. At each facility where sampling occurred, an attempt was made to collect soil samples from locations that maximize variety among parameters, such as slope, soil texture, soil moisture, geomorphic context, soil crusting, vegetation cover, bioturbation (mixing action of soil by burrowing rodents and other animals), soil mineral compositions, and physical and chemical properties (e.g., albedo, alkalinity, pH, and organic matter content). Sampling occurred three times (nominally winter, spring/summer, and summer/fall of 2017) at each facility over the course of the project to capture seasonal variation in activity and availability of *C. immitis*. Several transects were defined and revisited for periodic sampling, to compare the same locations over the course of a year.

In addition, we sampled several soil cores (approximately 30 cm depth) at sites that clearly support the growth of the pathogen as determined by data analysis of the first set of samples (collected in February 2016). Analysis of layers of soil (every few cm, resulting in 5 individual samples/core) reveal if *Coccidioides* are restricted to a certain soil depth.

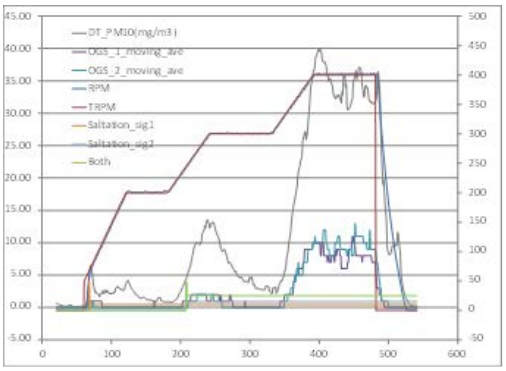
Soils so sampled were tested for the presence of *Coccidioides* spp. with a culture independent polymerase chain reaction (PCR) based method. Approximately 20 g of soil were collected at each sampling spot to allow the investigation of several replicates of each sample.

3.3 COLLECTION OF "WIND ERODIBLE" AND "ROAD" DUST SAMPLES

In conjunction with the collection of surface soil samples that was described above, suspendable dust samples were also collected at all sampling sites in the dry season. The DRI developed PI-SWERL® (Etyemezian et al., 2007; 2014; Sweeney et al., 2008, 2011) was used to generate windblown dust on test surfaces adjacent to where bulk soil sampling occurred.

The PI-SWERL® has been used extensively in prior wind erosion and windblown dust emission studies and provides a portable means to simulate the effect of high wind on soil surfaces. Dust emitted from within the PI-SWERL was sampled through a PM10 size inlet and subsequently onto a filter. The collected dust on the filters was tested for *Coccidioides* spp. using the same methods used to detect the pathogen in soils, see above. Material collected in the hopper of the size selective inlet (greater than 10 microns) was collected as well, and processed similarly, but separately (Figure 8).

In addition to sampling for the windblown component of dust with the PI-SWERL ®, a vehicle based mobile monitoring platform (TRAKER™, Figure 9, Kuhns et al., 2001; 2005; Etyemezian et al., 2006) was used to collect dust that is suspendable by travel on the unpaved roads of military facilities. Several samples of dust suspended in the wake of the TRAKER™ test vehicle was collected in one or more size fractions (PM10 and larger particles) for subsequent analysis in the dry season of 2017. The intent here was to determine if exposure to vehicle suspended dust is a pathway for exposure to arthroconidia not to try to pinpoint where *Coccidioides* spp. are present in the greatest quantity. Both PI-SWERL® and TRAKER™ have been used in prior SERDP projects (including the nearly complete RC-1729), and the latter is one of the methods that is listed as EPA OTM-34. Figures 10-15 show the locations where dust samples were collected. One of these figures (figure 14), shows the eroded landscape of the Antelope Acres area, and the changes in land use and erosion over time via satellite imagery.



Automated measurement cycle and example results

PI-SWERL measurement

PBS News Hour filming DRI and CSUB measuring dust emissions and sampling for Valley Fever spores at Edwards AFB

Figure 8. PI-SWERL® Has Been Used Extensively to Estimate Windblown Dust Emissions Including for other SERDP Projects (e.g., RC-1729).

The cylindrical chamber (top right) allows for recreating surface wind shear and measuring the resultant dust emissions (top left graph). The orange case contains filter sampling apparatus with PM10 and PM2.5 cuts.

Figure 9. TRAKER™ Was Developed as a Tool to Measure Emissions of Particulate Matter (PM10 and PM2.5) Because of Vehicular Travel on Unpaved Roads.

It has been used extensively in several SERDP projects (CP-1191, SI-1399, and RC-1729). The instrumentation on the back of the vehicle will be retrofitted to collect filter samples of suspended road dust for subsequent PCR and other analyses.

Figure 10. A. Several Dust Samples Were Collected via PI-SWERL at 4 Sampling Sites Northeast of NAS Lemoore. The Pathogen Could Not Be Detected Via Nested PCR in 3 of the Samples (green dots), But a Faint PCR Amplicon Was Obtained with Primer Pair EC3/EC100 from dust collected at the Edge of the Salt Pit (Orange Dot, PCR Product Was Not Sequenced [Not Strong Enough]). NAS Lemoore Can Be Seen in the Lower Left Corner of the Photo. B. Dust Collected from Site J Southeast of NAS Lemoore Was Positive and Confirmed Via Sequencing of the Obtained PCR Products (red dot) (see Methods for Details About Diagnostic PCRs). NAS Lemoore Can Be Seen in the Upper Left Corner of the photo.

Figure 11. Close-up View of Site J at the Intersection of Jackson Road and Highway 41 South of NAS Lemoore.

The sampling site is indicated with a red dot. The area shows extensive disturbance of surface soil by off-road vehicles, particularly east of the sampling site. Furthermore, it shows that an extensive part of this area has been converted to an agricultural field (lower left corner).

Figure 12. Two Dust Samples Were Collected Via PI-SWERL at Sampling Spots 6 and 7 West of Antelope Acres.

Dust collected at spot 6 was confirmed positive for Coccidioides spp. via nested PCR (red dot), and dust collected at spot 7 was negative for the pathogen (green dot) (see methods for details about diagnostic PCRs).

Figure 13. Sites 5 (left) and Site 6 (right) West of Antelope Acres and EAFB with Scattered Rabbit Brush and Salt Bushes.

Site 6 showed increased disturbance and erosion compared to site 5 with plenty of rabbit brush and scattered salt bushes.

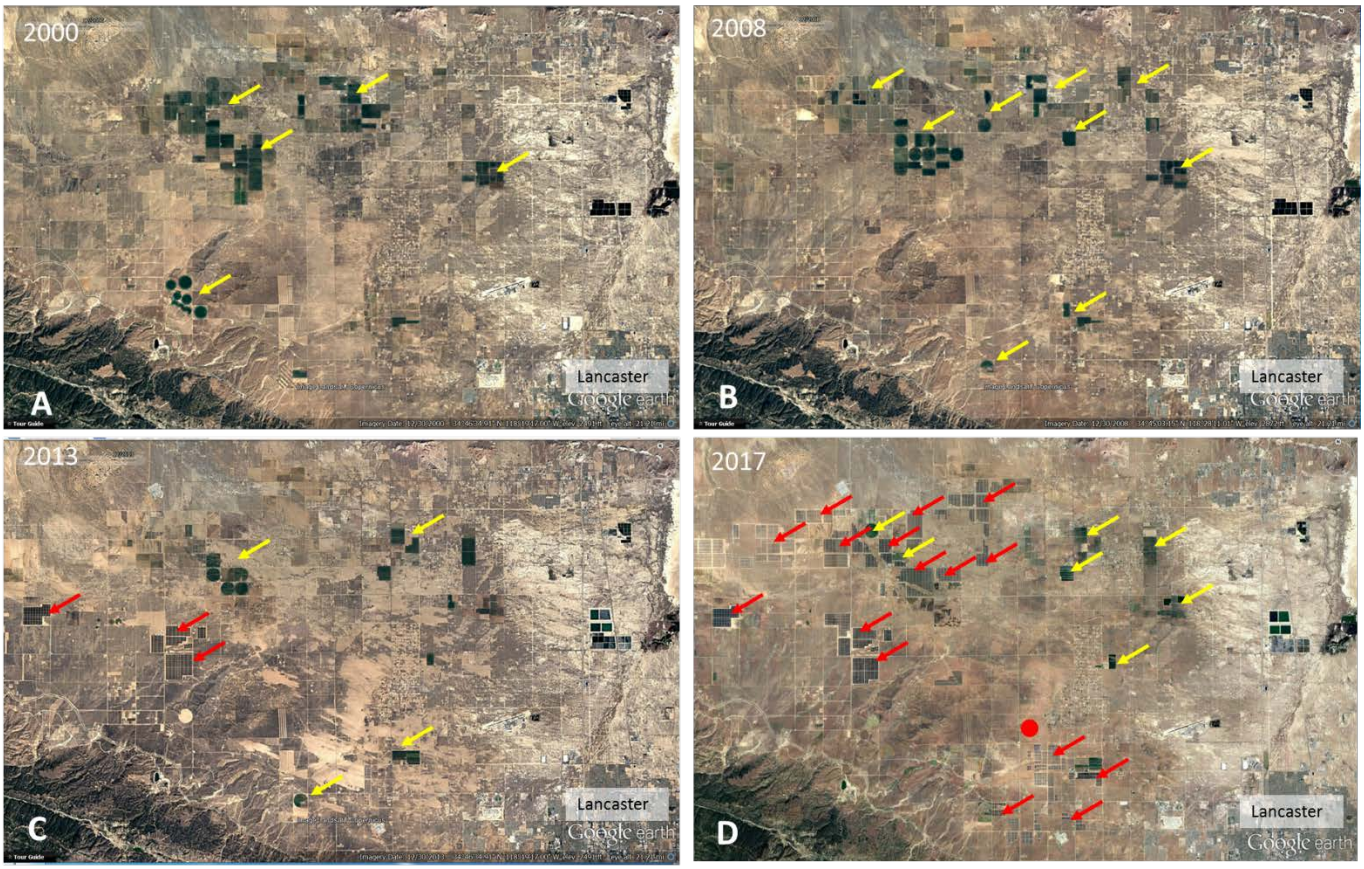


Figure 14. Satellite Imagery (Google Earth, Copernicus) Showing the Southwestern Mojave Desert in 4 Different Years, 2000 (A), 2008 (B), 2013 (C), and 2017 (D).

*The area shown is southwest of Edwards AFB. Yellow and red arrows indicate changes in land use over time: yellow arrows point to active agriculture, and red arrows point to renewable energy development, mostly utility-scale solar projects. Note the decrease of agriculture and the increase in renewable energy construction over time and the increased erosion in 2013 after the El Niño event in 2011/12 (**C**). The city of Lancaster can be seen in the right lower corner of the satellite images. The western edge of the Rosamond Dry Lakebed can be seen on the very right of the pictures, as well. The red dot indicates our sampling site west of Antelope Acres (**D**).*

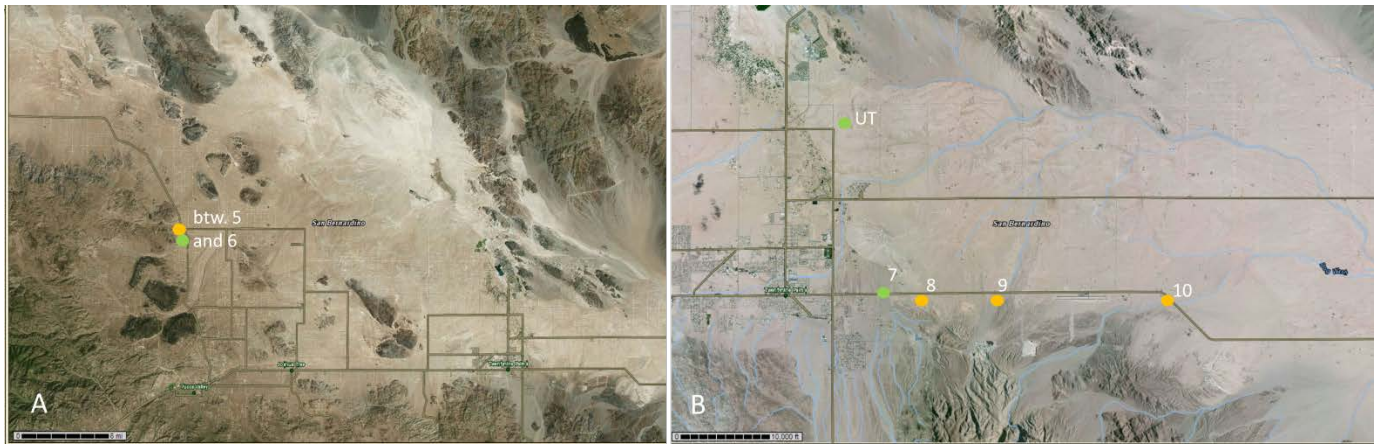


Figure 15. A. Two Dust Samples Were Collected Using the PI-SWERL West of Twentynine Palms Between Sites 5 and 6. One Sample Resulted in a Faint PCR Amplicon Using the Diagnostic PCR Method (orange dot), Another Sample Was Negative for Coccidioides spp. B. Additional Dust Samples Were Collected east of Twentynine Palms at sites 7-10 and at the northern tip of Utah Trail (UT). Samples that Resulted in a Faint PCR Amplicon with the Diagnostic PCR Are Indicated with Orange Dots, Negative Sites with Green Dots (see methods for details about diagnostic PCRs). A TRAKER Sample Taken Between Sites 7 and 10 Along Dirt Roads and a Dust Sample Collected at Site 5 Were Confirmed Positive for the Pathogen.

3.4 DNA EXTRACTION

The efficiency of the DNA extraction method from spores and conidia present in the soils was improved by adding steps to lyse arthroconidia and spores effectively which increased the sensitivity of the culture independent approach. Briefly, DNA was extracted from all soil samples (2 replicates) using the MoBio Powersoil DNA extraction kit (MoBio Laboratories, Carslbad, CA, USA) using the protocol provided by the manufacturer. However, prior to extraction, aliquots of soil were heated for 30 min at 70 °C and then incubated at 56 °C with Proteinase K (100 µg/mL) to enhance cell lysis from vegetative cells and dormant forms.

3.5 RNA EXTRACTION AND CDNA SYNTHESIS

It was initially intended to extract RNA from soil samples that appear *C. immitis* positive based on nested PCR (see method description above), using the RNA PowerSoil Total RNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) combined with the OneStep PCR Inhibitor Removal Kit (Zymo Research, Irvine, CA, USA). These analyses were undertaken but proved to be too resource intensive to complete within the resources of the pilot project. We believe that RNA analyses would be an important part of a larger, follow-on project.

3.6 NESTED PCR TO DETECT *C. IMMITIS*

We performed a nested PCR originally published by Baptista-Rosas et al. (2012) with modifications. This nested PCR included three individual PCR reactions to detect *Coccidioides* spp.

The first PCR uses primer pair NSA3/NLC2 to amplify a ~1,000 bp 18S rDNA fragment of all fungi, followed by a second PCR using primer pair NSI1/NLB4 which is specific to Ascomycetes and Basidiomycetes only (~900 bp). An aliquot of a 1:25 dilution of the second PCR was then used in two diagnostic PCRs performed with primer pair ITS1Cf/ITS1Cr (named VGITSf/r in this study) (~120 bp) (Vargas-Gastélum et al. 2015.) and with primer pair EC3/EC100 (~ 650 bp, Johnson et al. 2014) which are both more specific to *Coccidioides* spp. compared to the one originally suggested by Baptista-Rosas et al. (2012). A positive control used for all PCRs was obtained from *C. posadasii* Δchs5 strain (NR4548, BEI Resources).

The sensitivity and specificity of various published culture-independent PCR-based methods to detect *Coccidioides* spp. in soil and dust samples has been evaluated in our laboratory at CSUB. We also found the nested PCR method superior to a quantitative PCR method developed by Sheff et al. (2010) (data not shown).

3.7 PCR AND DGGE

To compare the fungal diversity in soil samples, we used a PCR amplification protocol with primer pair NS1/GC-fung which amplifies a ~350 bp fragment of the 18S rDNA gene based on the original protocol published by (May et al. 2001). This PCR was followed by DGGE (Denaturing Gradient Gel Electrophoresis) to obtain fingerprints of the fungal communities in the soil. In general, the DGGE method enables identification of dominant members of the fungal communities, which are visible as individual bands (also known as operational taxonomic units or OTUs) which can be identified by excising, re-amplifying and sequencing (Laragen, Culver City, CA). The DGGE method is widely used in environmental microbiology to identify dominant members of microbial communities. Advantages and disadvantages of the DGGE method compared to other fingerprinting methods are discussed in Okubo and Sugiyama (2009).

PCR amplification was performed in a C1000 Touch Thermal Cycler (BioRad, Hercules, CA, USA). DGGEs were performed with a DGGE system 2401 (CBS Scientific, Del Mar, CA, USA) using the protocol published by Hoshino and Morimoto (2008). DGGE bands can be excixed, reamplified, sequenced and then compared to 18S rDNA entries in the GenBank database using the Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov/blast, Johnson et al. 2008). The relatively short fragment size allows to identify the members of the fungal communities only to the family level, not to the species level. However, we did not attempt this approach, and rather opted to perform cloning and sequencing of larger PCR products from selected positive sites with additional funding to identify members of the fungal community to the species level by using a different primer pair.

The DGGE method is also useful for a combined DNA/RNA approach to distinguish active from dormant members of the microbial community and has been successfully applied by other researchers (Baldrian et al. 2012). Due to time constrains in this Limited Scope project, we were not able to pursue this task as originally planned but have started extracting RNA from some of the sites using the DNEasy RNA Powersoil DNA extraction kit. Extracted RNA was subsequently converted to cDNA using the SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase (Invitrogen) and stored at -80 C for future studies. (see Figure 16 for the position of different primer combinations on the rRNA gene).

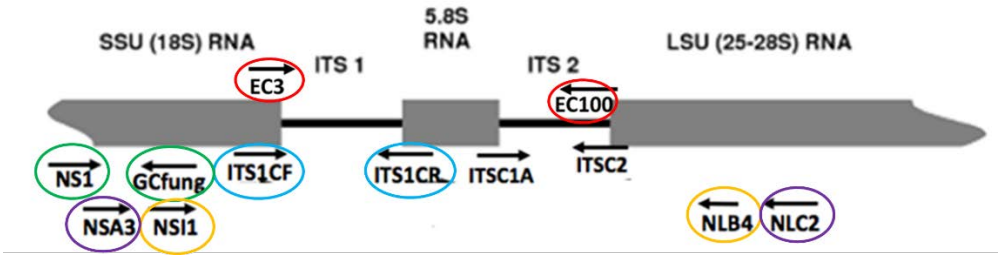


Figure 16. Position of Primers and Annealing Sites Used for Nested PCR Approaches to Amplify Fragments of the Fungal Ribosomal Gene (Hoegger and Kües 2007, updated).

The different primer pair combinations are indicated in assorted colors ITS= intertranscribed spacer regions).

3.8 ENVIRONMENTAL ANALYSES

Texture and ion chemistry were analyzed for soil samples using established techniques. Additionally, local site characteristics such as degree of disturbance and vegetative cover were noted in the field.

The percentage of sand, clay and silt in all soil samples (grain size analysis) was determined using the standard techniques of the DRI Soils Analysis Lab using a Malvern Mastersizer 2000 particle size analyzer. For pH measurements, we used a pH electrode (Oakton pH 510 series) and prepared a 1:4 ratio of soil:water to improve the fluidity of the slurry; suitable particularly for soils with high clay concentrations. Soil slurries could sit for 4 days to analyze total dissolved salts and electrical conductivity. Determination of redox potentials of the soils using soil slurries were not practicable because of a high fluctuation of the values over time towards more negative values. Highly aerobic desert soils should have redox potentials in the positive and not negative range. We also analyzed the inorganic ion composition in selected *Coccidioides* positive and negative soils from a subset of Coccidioides positive and negative samples using the standard ion chromatography techniques employed by the DRI Environmental Analysis Facility (EAF) lab using a Dionex ICS-3000 (Sunnyvale, CA) ion chromatograph. Results for fluoride, chloride, bromide, nitrite, nitrate, lithium, sodium, ammonium, sulfate, potassium, magnesium and calcium were obtained.

4.0 RESULTS AND DISCUSSION

In this section, we provide a summary of results that were obtained over the course of the pilot project. Additional detail and supporting information are provided in the Appendix to this report.

4.1 DETECTION OF *COCCIDIOIDES* IN SOIL AND DUST SAMPLES

Bulk soil samples were collected and analyzed for the presence of *Coccidioides* spp. from the three main sampling regions (NAS Lemoore, Edwards AFB, and Twentynine Palms MCAGCC) in late January/early February (winter samples), May/June (spring/summer samples), and early September (summer/fall). The first sampling event focused on a variety of different soil types and environments (5-7 cm depth only), whereas the second and third sampling events focused predominantly on *Coccidioides* positive sites, and only included re-sampling a subset of the negative sites (5-7 cm depth and core sampling). This sampling scheme allowed us to re-sample and analyze all *Coccidioides* positive sites throughout the seasons within the resources available for this pilot study.

We analyzed samples from 20 different locations around NAS Lemoore (n=56 [winter], n=31 [spring/summer], n=29 [fall], overall n=116), from 12 locations west of Edwards AFB (n=36 [winter], n=39 [spring/summer], n=19 [fall], overall n=94), and from 11 locations near Twentynine Palms MCAGCC (n=43 [winter], n=60 [spring/summer], n=76 [fall], overall n=177). The Twentynine Palms MCAGCC samples also included samples collected at three sites inside Joshua Tree National Park (Skull Rock, Hall of Horrors, and Hidden Valley). The spring/summer and fall sample sets contained several core samples (surface to ~30 cm depth in ~5 cm intervals) that were collected mostly from sites that tested positive for the pathogen in the winter (Edwards AFB: 7 cores from 5 sites, NAS Lemoore: 7 cores from 4 sites, Twentynine Palms MCAGCC: 14 cores from 10 sites including 1 from Joshua Tree NP). Overall, 389 soil samples were collected.

Positive diagnostic PCR products were sequenced to ensure that they indeed represent *Coccidioides* spp. Results from all diagnostic PCRs can be retrieved from tables A-D in the Appendix. Table 4 shows a summary of all diagnostic PCR results. This table also highlights the area around Twentynine Palms as a highly endemic area for the pathogen with 23% of the soil samples testing positive for *Coccidioides* with both diagnostic primer pairs in agreement. The area around NAS Lemoore which is dominated by agricultural activity, showed the lowest number of positive sites.

Table 4. Diagnostic PCR Results for All Soil DNA Extracts (n=389) (VGITSf/r and EC3f/EC100r).

	NAS Lemoore		**Antelope Acres (west of EAFB)**		**Twentynine Palms and Joshua Tree NP (south of MCAGCC)**	
	n	%	n	%	n	%
n	116	100	94	100	179	100
positive (with at least one diagnostic PCR)	32	27.59	14	14.89	76	42.46
positive (with both diagnostic PCRs)	**11**	**9.48**	**11**	**11.70**	**42**	**23.46**
negative (none of the diagnostic PCRs positive)	73	62.93	69	73.41	61	34.08

Soil core samples were collected from all three study areas in May/June and September/October. Not all sites could be sampled down to 30 cm depth because of underlying rocks. Table 5 shows results of the diagnostic PCRs for all soil core DNA extracts. *Coccidioides* was predominantly detected on the soil surface and in the upper soil layers. However, our results confirm that the pathogen can be found at all depths down to 30 cm.

Figures 17 to 19 display results obtained with diagnostic primer pairs targeting *Coccidioides* spp. in DNA extracts from soil and dust samples. It should be noted that the two different diagnostic primer pairs did not always agree. In fact, in only 54.78% of the PCRs did both reactions indicate the presence of the pathogen. This is likely due to incomplete homogenization of some of the soils, especially the moist ones collected during the winter, and the small amount of soil being used for DNA extraction (0.25g). For future work, we suggest combining DNA extracts from 4 individual soil aliquots to one DNA mix and to be more careful in ensuring a complete homogenization of the soils to ensure that all microbes from many different microhabitats in a soil sample will be mixed completely, e.g. through sieving soil samples that appear hard to break up using a 2mm sieve (Lee et al. 2009).

Table 5. Positive Diagnostic PCR Results for All DNA Extracts from Soil Core Samples (n=146) (VGITSf/r and EC3f/EC100r) in Percent.

		soil core sample and soil depth											
	n	**a** (0-2 cm)		**b** (5-7 cm)		**c** (10-12 cm)		**d** (18-20 cm		**e** (23-25 cm)		**f** (28-30 cm)	
		1*	2**	1*	2**	1*	2**	1*	2**	1*	2**	1*	2**
NAS Lemoore	26	11.5	7.7	11.5	3.9	7.7	3.9	7.7	3.9	0	0	3.9	0
Antelope Acres	40	0	0	0	0	2.5	2.5	0	0	0	0	0	0
Twentynine Palms	80	11.3	5	6.3	2.5	5.0	2.5	5.0	2.5	1.3	1.3	2.5	1.3

1* at least one diagnostic PCR positive

2** both diagnostic PCRs agree

Overall, PCRs with primer pair VGITSf/r which amplifies a short fragment of ~120 bp seemed to be slightly superior in sensitivity, compared to PCRs with primer pair EC3/EC100 which amplifies a ~650 bp amplicon. Of all 186 positive PCRs, the VGITSf/r PCR detected *Coccidioides* 101x (54.3%); the EC3/EC100 PCR detected the pathogen 85x (45.7%). Regarding specificity, the VGITS PCRs did not produce false positive amplicons. The ECf3/EC100r however, occasionally amplified DNA from unrelated soil fungi, most often *Cladosporium* spp. These false positive results are indicated in the tables as negative results and comprised about 4% of the amplicons that were sequenced. Even though the VGITSf/r primer pair seemed to be superior to the EC3f/EC100r PCR, a longer amplicon can be used for phylogenetic analyses, and allows the identification of the pathogen to the species level (Johnson et al. 2014).

In addition to soil samples, we also collected wind suspendable dust samples using the PI-SWERL and suspendable road dust using the TRAKER in the fall of 2017 from all three main sampling regions but concentrated in areas where *Coccidioides* spp. were detected in the soil (Table 6). Overall, we collected 4 wind suspended dust samples west of Edwards AFB, 13 from around NAS Lemoore, and 22 from near Twentynine Palms MCAGCC.

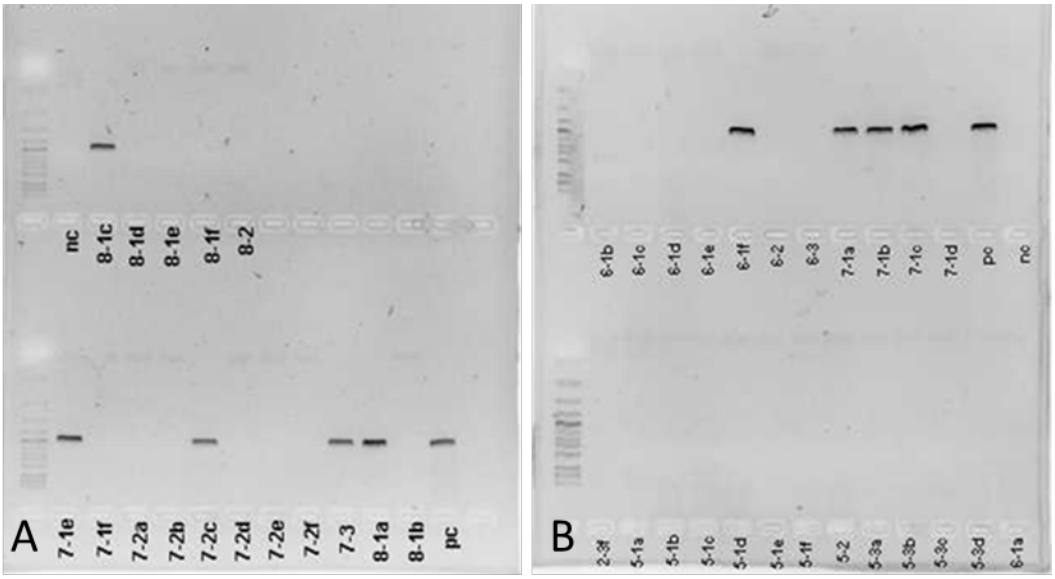


Figure 17. **A and B. Examples of PCR Results Obtained with Diagnostic Primer Pair EC3/EC100 (~650 bp) Using Soil DNA Extracts from the Area South of MCAGCC Twentynine Palms.**

A 100 bp DNA ladder can be seen on the left sides of the 2% agarose gels (PC=positive control, NC=negative control). All PCR amplicons were 97-100% related to C. posadasii (KR109218) entries in the GenBank nucleotide database.

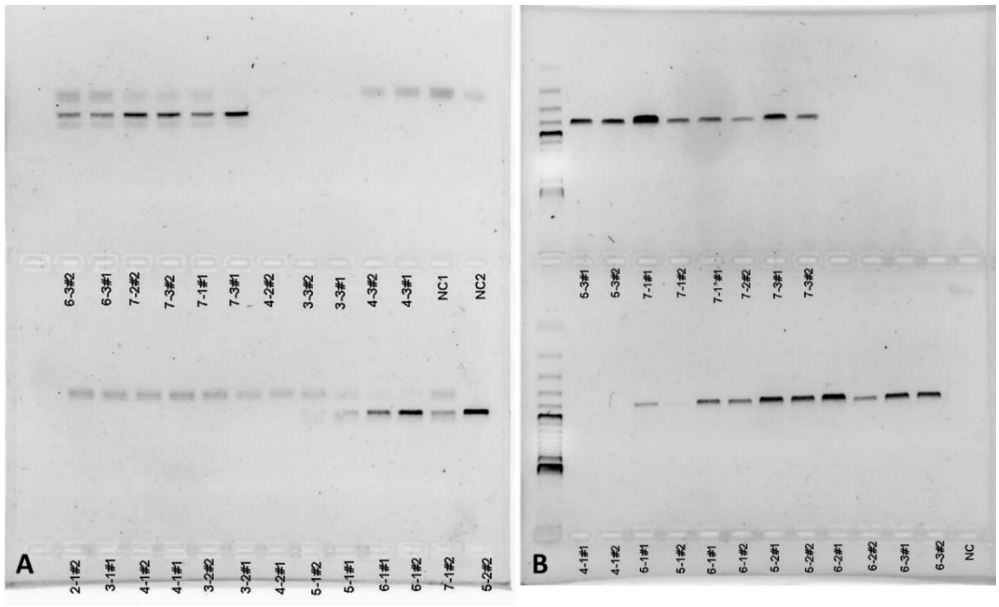


Figure 18. Examples of PCR Results Obtained with Both Diagnostic Primer Pairs Using Soil DNA Extracts from Antelope Acres.

*A 100 bp DNA ladder can be seen in the 2% agarose gel on the right (**B**) (PC=positive control, NC=negative control). **A**: Amplicons obtained with primer pair VGITSf/r (~120 bp), **B:** Amplicons obtained with primer pair EC3/EC100 (~650 bp). All PCR amplicons were 99-100% related to C. immitis (KY306695) or C. posadasii (KY399886) entries in the GenBank nucleotide database.*

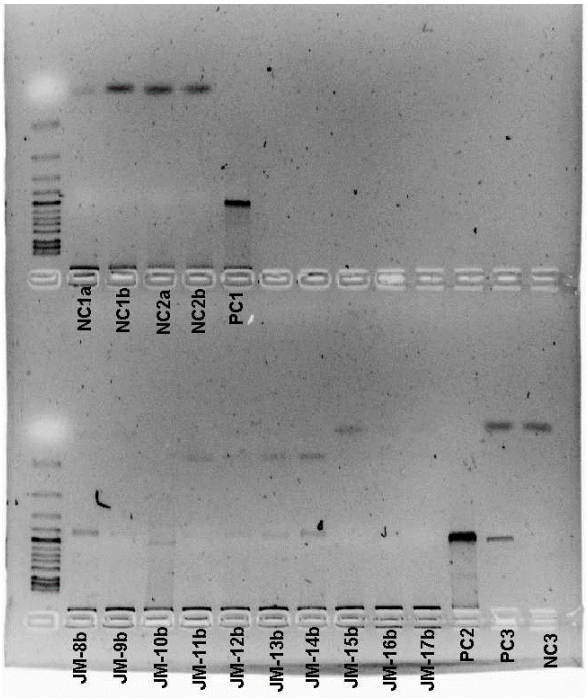


Figure 19. Examples of PCR Results Obtained with Primer Pair EC3/EC100 (~650 bp) Using DNA Extracts from Dust Samples Collected Near Twentynine Palms MCAGCC and (PC=positive control, NC=negative control).

Samples 10b and 15 b were TRAKER samples, all other samples were collected via PI-SWERL. Note that amplicons 9b and 10b were closely related to C. posadasii when sequenced and compared to entries in the GenBank nucleotide database (KY399886 99-100%). Samples 15b, 16b and17b were negative, and the remaining samples were either false positive, or more than one species contributed to an amplicon.

Assessing fungal species richness and diversity in soil and dust samples was performed using a PCR/DGGE approach that shows fingerprints of the fungal communities using a primer pair that amplifies a fragment of the fungal 18S rRNA gene. Species richness was generally low at all sites, with few dominant fungal species indicated by strong distinctive DGGE bands. Fungal diversity was not even throughout depths and samples collected along transects or several meters apart from each other displayed variations in diversity. The pathogen appeared to be in some of the fingerprints, but rarely appeared as a dominant species. Note, that the pathogen only appears on the DGGE gel if it is a dominant member of the fungal community. Therefore, samples that indicated the presence of the pathogen using a nested PCR approach including a diagnostic primer pair might not show the pathogen in the DGGE fingerprint which is a non-nested, less specific method. A nested PCR allows the detection of a minority species that would otherwise omit detection. Amplicons from minority species in a non-nested PCR such as the one used for DGGE profiling will likely diffuse into the background. DGGE bands have to be excised, re-amplified and sequenced to confirm if they indeed represent the pathogen, because PCR fragments with the same quantities of different nucleotides (A, C, G and T) but in different arrangement will denature in the same melting area of the DGGE gel. Re-amplification and sequencing of DGGE bands was not attempted in this pilot study.

Table 6. Detection of Coccidioides sp. in Wind-suspendable and Vehicle-suspendable Dust Samples Collected Around NAS Lemoore, Antelope Acres (west of EAFT) and MCAGCC Twentynine Palms (n=14 [PISWERL], n=6 [TRAKER]).

See also detailed table D in Appendix A.

Site	Sample Type	Soil Type and Map Unit	Coordinates	Diagnostic PCR EC3/EC100 (neg/pos)
Lemoore				
21. street to north of Jackson Ave., Lemoore	TRAKER (YUCTQ173)	Panoche clay loam, saline-alkali (151)		neg
site 1, N-26, along dirt road	TRAKER (YUCTQ177)	Panoche clay loam, saline-alkali (151)	36.37695, -119.93247	neg
site 1, N-26, dirt road	PI-SWERL (YUCTQ176)	Panoche clay loam, saline-alkali (151)	36.37695, -119.93247	neg
site 2, dry-lake	PI-SWERL (YUCFQ174)	Panoche clay loam, saline-alkali (151)	36.37172, -119.93171	neg
site 3, near creek, saltbush area	PI-SWERL (YUCFQ173)	Vanguard sandy loam/Gepford clay (partially drained), (168/115)	36.25576, -119.83454	neg
site 4, iodine bush area	PI-SWERL (YUCFQ177)	Lemoore sandy loam/Boggs sandy loam (partially drained), (137/103)	36.25467, -119.80995	**pos**
21. street, Lemoore to Bakersfield, near intersection to Hwy. 58	TRAKER (YUCFQ172)			neg (but showed a band that could represent the pathogen on the DGGE)
Antelope Acres				
site 7	PI-SWERL (YUCTQ171)	Adelanto coarse sandy loam (CaA)	34.74670, -118.30689	neg
site 7	TRAKER (YUCTQ171)	Adelanto coarse sandy loam (CaA)	34.74670, -118.30689	neg
site 5	PI-SWERL (YUCTQ175)	Cajon loamy sand (CaA)	34.73926, -118.30379	**pos**
dust on car after sampling (site 8)	swab		34.74748, -118.3047	neg
Twentynine Palms				
site 5	PI-SWERL (YUCFQ179)	no data	34.26574, -116.44589	**pos**
site 6	PI-SWERL (YUCTQ179)	no data	34.25917, -116.44067	neg
site 7	PI-SWERL (YUCTQ178)	no data	34.13676, -116.01933	neg
site 8	PI-SWERL (YUCFQ182)	no data	34.13583, -116.00295	**pos**
site 9	PI-SWERL (YUCFQ178)	no data	34.13607, -115.97171	**pos**
site 10	PI-SWERL (YUCFQ181)	no data	34.13433, -115.9116	**pos**
dirt roads between sites 7-10	TRAKER (YUCTQ182)	no data		**pos**
site UT	PI-SWERL (YUCTQ172)	no data	34.19569, -116.0364	neg
site UT	TRAKER (YUCTQ172)	no data	34.19569, -116.0364	neg

Overall, the diversity of fungi in soil samples appears to be very low in samples from agricultural fields, likely because of broadspectrum antifungals being used to inhibit the growth of fungal plant pathogens which will also affect harmless fungal saprophytes in the soil. Fungal species diversity was highest in 5-10 cm depth indicated by an increase in DGGE bands in the DGGE profiles. Figures 10-13 in the Appendix show examples of DGGE profiles of the fungal communities in soil samples collected from different sites.

DGGE fingerprints of dust samples collected with PI-SWERL and TRAKER revealed a low diversity of fungal species in general (Figure 20). Additionally, different locations show different fungal profiles, but similarities in the fingerprints indicate the likely presence of some fungal ubiquists that were found in almost all samples (as can be seen by the double band in the center of most profiles).

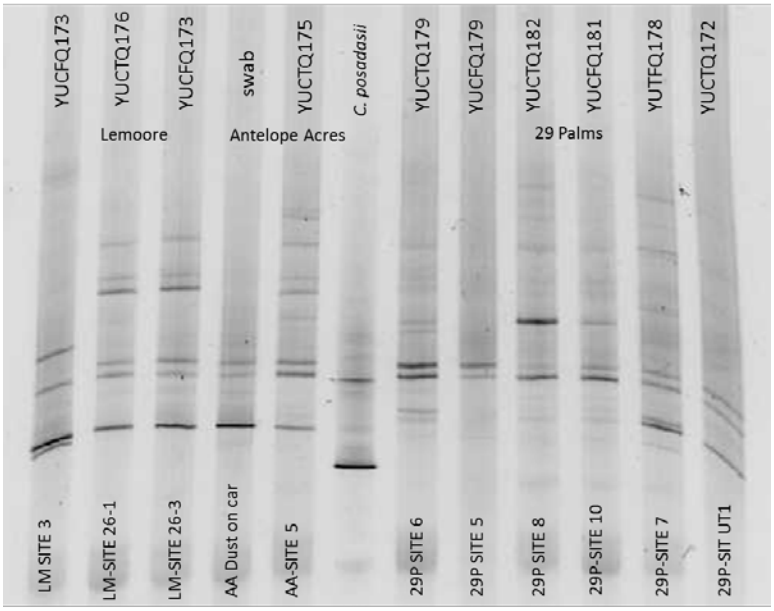


Figure 20. DNA Fingerprints of Fungal 18S rDNA from Dust Samples Collected Near Lemoore, Antelope Acres and Twentynine Palms (summer 2017) Reveal the Presence of Different Fungal Species in Fugitive Dust.

The overall diversity was low showing between 2 and 8 individual bands that represent different Operational Taxonomic Units (OTUs). An 18S rDNA amplicon of C. posadasii can be seen in lane 6 (from the left). The pathogen appears to be absent (or under the detection limit of the DGGE method) in these dust samples as no band in the same melting area as the C. posadasii amplicon was observed in any of these dust samples.

4.2 RESULTS IN SUPPORT OF OBJECTIVE I: DETERMINE IF THERE ARE ANY MEASURABLE SOIL-, OR ENVIRONMENTAL VARIABLES OR COMBINATIONS THEREOF THAT ARE PROMISING AS PROXIES FOR THE PRESENCE OF LIVE *COCCIDIOIDES* SPP. IN SOILS.

4.2.1 pH, electrical conductivity and total dissolved salts

The analyses of soil environmental parameters over the seasons revealed quiet distinct soils at each of our sampling locations. Averages of these parameters for *Coccidioides* positive and negative sites are displayed in figures 21 and 22. The results revealed significantly higher pH values in soils around NAS Lemoore and Twentynine Palms compared to Antelope Acres west of Edwards AFB. Electrical conductivity (mS/m^3) correlated with total dissolved salts (g/L) and was highest in soil samples collected from Lemoore. Seasonal variation of soil parameters was observed as well for all sites.

Our results indicate that *Coccidioides* spp. can be detected in somewhat different environments with most of the positive soils showing a pH range between 7.1 – 8.1. The pathogen is also halotolerant, because it was detected in highly salty environments with elevated electrical conductivity east of NAS Lemoore. These sites where characterized by the dominance of salt bushes (*Atriplex* spp.) or iodine bushes (*Allenrolfea occidentalis*), which are indicator plants of sandy, often salty, distinctly alkaline soils, such as desert washes and saline dry lakebeds or seasonally flooded areas (see also 4.1.1.4).

Overall, sites near NAS Lemoore and EAFB are characterized predominantly by sandy loams, whereas sites in the Twentynine Palms area south of the MCAGCC are mostly loamy sands.

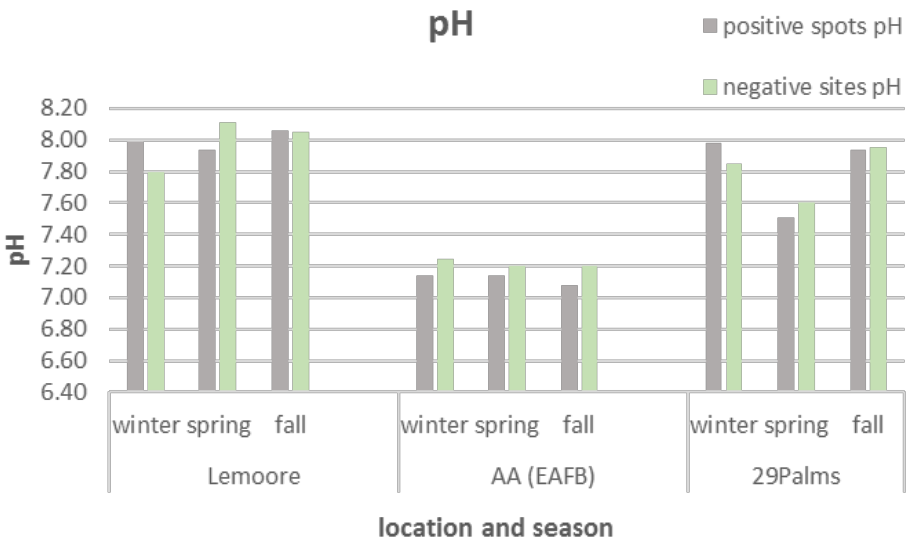


Figure 21. Averages of pH Values for Coccidioides Positive and Negative Soil Samples Over the Seasons.

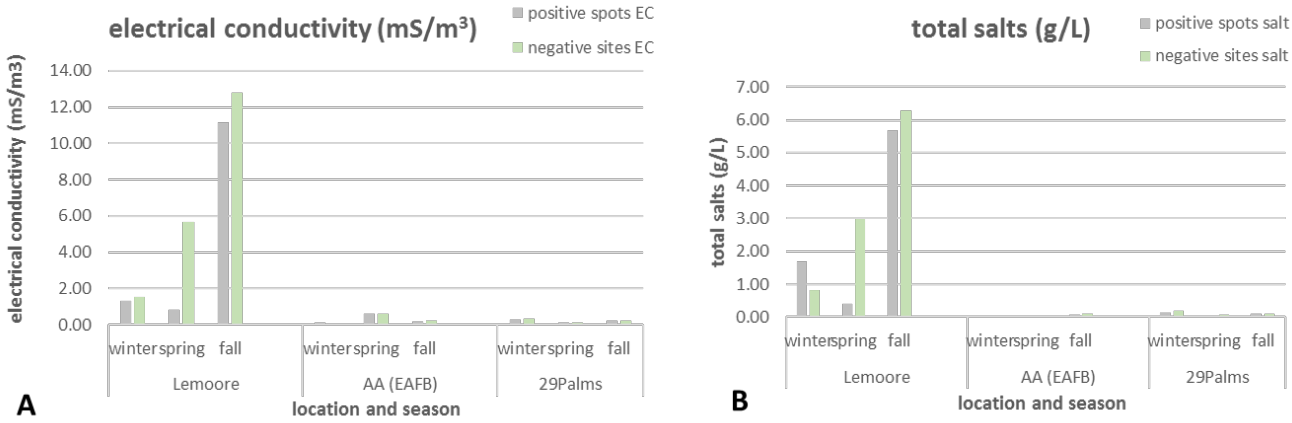


Figure 22. Averages of Electrical Conductivity (mS/m^3) and Total Dissolved Salts (g/L) for Coccidioides Positive and Negative Soil Samples Over the Seasons.

We did not find a significant difference for soil pH and electrical conductivity comparing sites where the pathogen was detected and sites where the pathogen seemed absent, looking at the entire year and the different seasons. The p-values ranged from 0.170-0.870. The data were normalized because more negative sites than positive sites were detected. The calculations were performed by using the program excel, one-way ANOVAs.

4.2.1.1 Grain size analysis and CaCO3 content

In addition, we analyzed grain size and $CaCO_3$ content for a subset of soil samples and found that *Coccidioides* positive soil samples had a slightly higher content of silt and clay, and a slightly higher $CaCO_3$ content compared to *Coccidioides* negative sites. However, the range of values for positive and negative sites overlap greatly, indicating that a wide range of these parameters is tolerable. Soils from different sampling regions exhibited larger differences than positive and negative sites within the same region. In general, samples collected in the Mojave Desert (Twentynine Palms area) were sandier and less silty and contained slightly higher levels of $CaCO_3$ compared to samples collected in the Central Valley around Lemoore (Tables 7, 8).

Table 7. Comparison of Grain Sizes and $CaCO_3$ Content from Selected Coccidioides Positive and Coccidioides Negative Sites in Percent.

	GRAVEL - % -	$SAND_{FE}$ - % -	$SILT_{FE}$ - % -	$CLAY_{FE}$ - % -	CaCO3 - % -
Coccidioides negative sites (n=22)					
Average	4.9	61.6	31.5	3.9	0.6
Median	4	61.6	31.3	3.9	0.1
Range	0.00-30.2	30.44-76.05	10.53-66.8	1.22-9.74	0.00-3.44
Coccidioides positive sites (n=15)					
Average	9.1	57.4	38.4	4.2	1.3
Median	10	61.1	34.9	3.3	0.6
Range	1.4-16.7	29.1-76.31	20.7-66.19	1.72-16.11	0.10-8.8

Table 8. Variation of Grain Sizes and $CaCO_3$ Content in Soil Samples collected around Twentynine Palms, Joshua Tree NP, Antelope Acres (west of EAFB) and around NAS Lemoore.

	>2mm Fraction	<2mm Fine-earth Malvern			
	GRAVEL	$SAND_{FE}$	$SILT_{FE}$	$CLAY_{FE}$	$CaCO_3$
	- % -	- % -	- % -	- % -	- % -
29 Palms (n=14)					
Average	8	69.36	27.33	3.31	0.65
Median	7.5	71.9	26.1	2.7	0.61
Range	2.4-12.1	54.67-76.05	20.1-35.58	1.4-9.74	0.37-1.34
Joshua Tree (n=5)					
Average	13	55.9	35.7	3.1	0.11
Median	10	60.6	36.8	2.7	0.14
Range	6.2-30.2	37.69-66.12	31.59-57.56	2.3-4.75	0.00-0.14
Antelope Acres (n=15)					
Average	3.9	62.3	30.6	3.6	0.05
Median	3.6	61.8	31.2	3.8	0.03
Range	0.2-8.4	51.06-76.04	10.53-44.01	1.22-6.24	0.00-0.15
Lemoore (n=7)					
Average	4	39.7	53.7	6.6	3.51
Median	1.4	30.4	59.5	4.7	3.44
Range	0.00-12.3	24.44-60.99	32.74-66.8	3.21-16.11	0.84-8.8

Soil environments are characterized by soil physical and chemical parameters. These parameters are influenced and also influence the diversity and dynamics of both the soil microbiota including *Coccidioides* spp., as well as vegetation. The soil parameter information available in the USDA websoilsurvey database, although averaged over large spatial scales, is useful to distinguish environments from each other. In the search for suitable soil parameters that may indicate a habitat that can support the growth of *Coccidioides* spp., soil parameters from sites where the pathogen was detected can be compared to sites where it was not detected. The observed uneven distribution (both horizontally and with depth) of the pathogen and other microorganisms in the soil (microhabitats) makes it difficult to determine exact supportive soil conditions, especially because soil conditions change over the seasons, and the presence or absence of antagonists to the pathogen (biological soil parameter) which might play a very important role is not practicable to assess on a larger scale (Chaparro et al. 2012). The complex interactions among soil microbes and variations in key factors such as plant species and soil parameters determining soil microbial diversity has been reviewed by Garbeva et al (2004). Soil parameter data for these sites are presented in the Appendix figures 1-9.

Based on our findings an increase in pH, electrical conductivity, total dissolved salts, silt and clay content can be helpful in describing a suitable *Coccidioides* habitat to some degree, but these do not appear to be first order parameters in controlling the occurrence of the fungus. There is inferential evidence that microbial antagonists to the pathogen and the type of organic matter in the soil may have an influence. So that, for example, a saline-alkaline soil, with enough soil moisture during winter and spring might present a suitable habitat for the pathogen, but it will be absent if antagonists reside or when needed nutrients are scarce or not available. In that sense, the range of soil parameters measured provide an envelope of necessary conditions for the growth of *Coccidioides*, but do not prescribe the conditions that are sufficient for the fungus to thrive. One important finding is that the fungus is nearly entirely absent from agricultural fields, suggesting that once lands have been severely disturbed from their original state, the resulting degraded land becomes unsuitable for fungal growth. This is an important observation to keep in mind as we discuss the nexus of locations where wind erosion is likely and where *Coccidioides* is available for resuspension.

4.2.2 Ion chromatography

NAS Lemoore is situated in an agricultural area, where little natural vegetation remains. Use of fertilizer and pesticides is common which is mirrored by the results of ion chromatography with increased levels of nitrite and sulfate in particular. Ion chromatography also revealed increased levels of bromide (~100 µg/g of soil) at this site and at other more natural sites in the Lemoore area in contrast to sites in the Mojave Desert. Furthermore, ion chromatography revealed that soils from the Lemoore area were heavily influenced by agricultural activities as indicated by high amounts of nitrates and sulfates in contrast to desert soils which were rich in calcium and potassium. Soil samples from Antelope Acres and from the Mojave Desert around Twentynine Palms show high levels of soluble potassium and calcium, likely originating form soil parent material such as granite and basalt, with many samples showing levels higher than 100 mg/g. Interestingly, according to the California Department of Pesticide Regulation (CDPR), potassium bicarbonate and potassium silica are known antimicrobial agents that are being used to fight some plant pathogens (http://www.cdpr.ca.gov/docs/cannabis/can_use_pesticide.pdf , see also Ordóñez-Valencia et al. (2009) and Shen et al. (20210)). If *Coccidioides* is resistant to these chemicals, it would hint towards an advantage over other soil fungi and explain why the highest amount of positive soil samples were found in the Mojave Desert. Increased levels of calcium and potassium were also detected in soils from Antelope Acres but were very low or absent in agricultural soils from Lemoore (see figures 14-17 in the Appendix).

4.2.3 Land use change and plants as indicators for Coccidioides habitat

This study revealed several hot spots where the pathogen was detected in which differed regarding degrees of disturbance, vegetation and land use.

Agricultural activities such as ploughing and harvesting result in regular dust emissions in the spring and fall seasons near NAS Lemoore. The natural vegetation of the Central Valley has been significantly changed towards industrialized agriculture. Few remaining semi-pristine habitats remain, which is especially evident in the area around Lemoore (Germano et al 2011). In this area, the pathogen was detected in soils from sites that showed remaining native vegetation, and alkaline-saline soils, located northeast of the base in winter and spring. Interestingly, all samples collected from agricultural fields or orchards were negative.

One site that was revealed as a potential growth site of the pathogen in the Lemoore area was site J, South of NAS Lemoore. Site J is located near the intersection of Jackson Road and Highway 41 with daily traffic connecting Lemoore and Stratford (Kings County). The sandy loams in this area also show substantial disturbance by off-road vehicles, a potential dust-hazard in the dry season that increases the risk of being exposed to arthroconidia of the pathogen for humans and animals in this area. Airborne arthroconidia from a site like this can also travel by the wind to other locations including NAS Lemoore. Satellite imagery (google earth, landsat) reveals that the general area Southeast and East of NAS Lemoore is composed of salty sandy loams. However, most of this land has been converted to agricultural fields over time.

Soils from site J are composed of sandy loams (landform: alluvial fans and rims on basin floors), are highly-saline with elevated pH, have an elevated electrical conductivity and total dissolved salts, and are also named alkali-sinks. plants, such as California native *Allenrolfea occidentalis* (iodine bush) were the dominant plant species present. This plant indicates a riparian wetland.

The distribution of the species throughout California can be seen on an interactive map provided by Calflora.org, https://www.calflora.org/cgi-bin/species_query.cgi?where-calrecnum=171. This plant could be an indicator species of a suitable habitat for *Coccidioides*. Its distribution correlates with known and suspected hotspots of the pathogen such as Kern County and Northern Los Angeles County, as well as locations near the state prisons Avenal and Pleasant Valley in Freson County, in in some areas close to Twentynine Palms. However, more areas like this must be investigated for the presence of the pathogen to confirm this statement because this plant species is also established in in non-desert mountainous or coastal areas (Germano et al. 2011).

The area near Antelope Acres west of EAFB is characterized by either grassland or shrubland, both highly disturbed and eroded. Illegal sheep grazing is common. The area is also experiencing a boom in construction for renewable energy, manly solar plants. Dust emissions from this highly eroded land that experiences high wind, are common and probably are what have led to an increase in coccidioidomycosis in humans over the last decade. We found *Coccidioides* spp. present in soil samples collected in February 2017 in all samples from sites 5-7, and 1 out of 3 samples was positive for sites 1 and 4. The pathogen was also detected in May, but only at sites 5 and 7. We could not detect the pathogen in soils collected at this site in September. During the May sampling event 5 core samples were collected from site 2 and 7 (2 cores each) and one core from site 5. The 2 positive samples were collected from site 5 (5-7 cm depth) and from one of the cores collected at site 7 (also 5-7 cm depth). The diagnostic PCRs for all other core samples were negative.

One parameter that was not investigated in the present study but that has promise as a determining factor in whether or not a growth site is suitable, is the type of organic matter that is present in the soil. As a species that is less able to biodegrade plant derived organic matter, and preferentially uses animal derived matter such as keratin, high rodent abundance could be included as an indicative parameter (Sharpton et al. 2009, Li et al. 2014).

4.3 RESULTS IN SUPPORT OF OBJECTIVE II: DETERMINE IF THE SUSCEPTIBILITY OF A SOIL WHERE *COCCIDIOIDES* SPP. ARE PRESENT TO WIND EROSION AND DUST EMISSION CAN BE RELATED TO THE AMOUNT OR EXISTENCE OF SUSPENDED ARTHROCONIDIA.

We were able to detect the pathogen via diagnostic PCR in several dust samples collected with the PI-SWERL method (See Table 6). In addition, DGGE fingerprints of the fungal communities in spores also revealed the presence of the pathogen in some of the samples that were confirmed positive via diagnostic PCR. A fungal species that shows up as an Operational Taxonomic Unit (OTU) in the DGGE profile is a dominant member of the community. However, other fungal species were more abundant in DGGE profiles than *Coccidioides*, although it appeared to be among the dominant members of the microbial community in some of the soil samples, especially around Twentynine Palms. We reiterate that the PI-SWERL samples were only collected from sites that had previously tested positive for the presence of *Coccidioides.*

The data collected are limited because sample collection with the PI-SWERL is somewhat resource intensive and had to be limited due to the pilot nature of the study. Additionally, since it was not possible to complete the RNA analysis, it was also not possible to determine if there is a relationship between where the suspendable surface material was found to be positive and where the fungus has live growth sites. Given the experience we obtained, we expect that these are limitations that can be overcome with greater human and material resources than were allocated for this pilot project.

The fact that some DGGE profiles from environmental samples show a band in the same melting area as *Coccidioides* is an indicator for a potential growth site of the pathogen that can be confirmed by an RNA analysis.

The above caveats aside, it is interesting that most of the sites that tested positive for the presence of *Coccidioides* with a bulk surface sample tested negative for material that was resuspended from the surface of those same sites using the PI-SWERL. At NAS Lemoore, the only site where the wind-suspendable dust tested positive (LM-4, also named site J) was from an area where a degraded, but vegetated patch of land meets a roadway and is surrounded by agricultural fields. Similarly, around Antelope Acres (upwind of Edwards AFB), AA-5 tested positive for wind-resuspendable dust. This site was on a degraded landscape that was surrounded by areas that had been graded at some point prior and appear to be slated for solar power infrastructure installation. These two facilities had reported relatively high levels of valley fever illness (See Figure 3) in prior years.

Twentynine Palms MCAGCC had relatively few reported cases of valley fever over the same time period. It also had the highest number of PI-SWERL-resuspended dust samples that tested positive for *Coccidioides.* Those were around 29P-5, 29P-8, 29P-9, and 29P-10, all areas that were vegetated with mostly creosote bush with some other shrubs and some grasses. Although resuspended dust samples were not collected from there, several bulk samples from within Joshua Tree National Park, presumably among the least disturbed landscapes one will find due to its protected status, also tested positive.

4.4 RESULTS IN SUPPORT OF OBJECTIVE III: DETERMINE IF EXPOSURE TO DUST SUSPENDED FROM TRAVEL ON UNPAVED ROADS IS RELATED TO EXPOSURE TO ARTHROCONIDIA

In general, it was more challenging to collect sufficient sample material from sampling road dust with the vehicle-based system than wind-suspendable dust from PI-SWERL. This has to do with how the two instruments work and how samples are collected, so that this observation should not be taken as an indicator that wind erosion plumes are somehow more intense than road dust plumes. It is just that sample collection with the TRAKER takes place in a part of the plume that has undergone more dilution than in the case of the PI-SWERL. Road dust samples were collected over several miles of roadway travel, so that they do represent an average sample over spatial scales that are in some sense much more representative than can be collected with point measurements using the PI-SWERL.

At NAS Lemoore and Antelope Acres near Edwards AFB, the two associated road dust samples were negative for *Coccidioides*. At Twentynine Palms, the road dust sample that was collected from roads in the area surrounding sites 29P-7, 29P-8, 29P-9, and 29P-10 was positive for both primer pairs. It is difficult to place this limited information into any kind of quantitative assessment of exposure risk. This is especially true because a positive DNA test does not provide any information about whether the genetic material is accompanied by any risk of infection or if it is just extraneous and not really viable in a human host. At the simplest level, these findings suggest that simply traveling on unpaved roads in endemic areas is a potential pathway for exposure. Given the extensive exposure to road dust that troops are subjected to during training activities (especially when in convoys), exposure from road dust may be one of the most prevalent pathways to infection.

4.5 RESULTS IN SUPPORT OF OBJECTIVE IV: IDENTIFY PROMISING PARAMETERS THAT CAN BE LINKED TO LARGE-SCALE DATASETS, MAPS, AND SATELLITE RETRIEVALS FOR THE PURPOSE OF SCALING FIELD RESULTS UP TO THE INSTALLATION- OR REGIONAL-SCALE.

Our preliminary findings indicate that pH, electrical conductivity and total salts alone can vary over a relatively large range and cannot be used as especially specific indicators of suitable habitats that allow *Coccidioides* species to grow. However, there is indication that an increase in fine particles (silt and clay), elevated $CaCO_3$ and soluble Ca and K in soil could be used as a marker for higher probability of *Coccidioides* habitats. The presence of sparse growth of xerophytes such as *Atriplex* spp., *Allenrolfea occidentalis*, *Larrea tridentata* can indicate these soil conditions and are helpful for the investigator to determine sampling sites. As discussed in the Conclusions section, large scale datasets that delineate where possible growth sites for *Coccidioides* border human disturbance activities could be the key to expanding results from specific studies to the regions in and around the DoD facilities in endemic areas.

5.0 CONCLUSIONS AND IMPLICATIONS FOR FUTURE WORK

The association of positive sites with shrub-vegetated desert surfaces, albeit clearly impacted by human activity, and the clear exclusion of *Coccidioides* growth sites from agricultural lands suggests a different paradigm for exposure than we had originally hypothesized. We had originally envisioned that the growth sites of *Coccidioides* were peppered through a landscape but covered a rather small areal extent. When these areas are encountered incidentally (for example, while walking during training exercises), arthroconidia that are readily available at the surface are suspended and can be inhaled. When vegetation is removed from these areas then the surface becomes easily erodible by wind, so that these small patches of growth areas become sources of airborne arthroconidia.

The preliminary results of the present study have refined our conceptual understanding of the exposure pathway. It appears that the *Coccidioides* growth sites are likely restricted to areas where vegetation such as creosote and salt bush are still in place. This is true for the sampling sites around NAS Lemoore, Edwards AFB, and Twentynine Palms MCAGCC. Perhaps this is because those areas are preferred by rodents, and the fungus prefers to feed on rodent keratin than plant detritus. When the landscape is reworked to the point of becoming an agricultural field, the soil is apparently no longer hospitable, either due to lack of the right combination of organic material, the application of chemicals that inhibit fungal growth, the physical disruption caused by frequent soil disturbance such as tilling, the establishment of microbes that act as antagonists to the pathogen, or some such combination of factors.

Related to this, it appears that when the soil surface is relatively intact, and the surface crust is not disturbed, the potential for exposure is relatively low. This is supported by the fact that PI-SWERL testing on sites that were known to be positive for the fungus (based on bulk samples collected from the first few cm) resulted in suspended dust samples that were largely negative except from locations that were along the boundary of a vegetated area and a non-vegetated area where the potential for wind erosion is much higher. This seems to suggest that the same parameters that make a location hospitable for the growth of *Coccidioides* (shrubs, vegetation, rodents, crust) are also parameters that on balance reduce the wind erodibility of the surface. If true, this might suggest that simply standing in a landscape where *Coccidioides* is growing over a large areal extent may not be much of an exposure hazard. Of course, there is certainly some exposure hazard because presumably the fungus relies on the aeolian transport of arthroconidia to propagate itself, but surprisingly, this might be a relatively minor pathway for exposure.

The majority of positive samples obtained with the PI-SWERL were from sampling locations that were at the overlap area between vegetated (albeit heavily degraded) surfaces and vegetation-free surfaces such as unpaved roads and graded fields. These observations suggest that maximum risk of exposure happens at or near the point and time of land disturbance. In the absence of disturbance, the risk of exposure from wind erosion appear to be mitigated by the relatively resilient surfaces of growth sites. An additional point is that areas where there is vegetation, the soil surface is protected from wind erosion because the vegetation acts as a shelter through the process of shear-stress partitioning.

With this in mind, we expect that exposure can happen in the immediate vicinity of land disturbance activity at the time of the activity. This can include activities such as trenching, grading, traveling on the surface with tracked vehicles, or any activity that significantly abrades or punctures the soil crust to the point that inhalable arthroconidia are physically lifted out of and around growth sites into the air in the vicinity of humans. This is likely the type of exposure that one would experience during varied activities such as construction (e.g., solar plants), driving tent stakes into the ground, and walking behind a tracked vehicle as it travels on a relatively undisturbed surface for the first time.

We can also expect that exposure happens over a more diffuse area during wind erosion events and travel on unpaved roads. In one case, the source areas are freshly disturbed surfaces such as very recently graded land where the portions of the fungus are still viable. These types of source areas are likely temporary since either the landscape becomes more stable over time and perhaps suitable as a (non-erodible) *Coccidioides* growth site or the fungus simply cannot survive and eventually is no longer a major exposure hazard. As an aside, one has to consider though, that it is not known how long dormant arthroconidia of the pathogen remain infective in soils that do not support its growth anymore. In the other case, exposure is from erosion of areas that are adjacent to vegetated growth areas, where the fungus has spilled over into the erodible surfaces by wind, water, or animal transport. These types of source areas are potentially much longer lived since the vegetated growth area can continue to supply viable fungal material as long as the *Coccidioides* is able to survive. Erosion in this case can be defined more generally as by wind or by the action of vehicle tires and tracks on the surface of an unpaved road.

This conceptual model for exposure suggests that there is probably little value in trying to identify soil conditions that are ideally suited for *Coccidioides* growth as those are present through the endemic area and as the fungus is probably much more ubiquitous than we had originally expected. The growth areas can be limited slightly by excluding especially inhospitable conditions. However, from the perspective of exposure, the conceptual model presented suggests that areas of greatest interest are very near the overlap of landscapes vegetated with creosote and salt bush and areas that have been completely converted to use by human activities. These include borders between farmland and desert landscapes, on roads that go through vegetated areas (including highways immediately after rainfall events), and in drainages following precipitation events at the point that the drainage starts to dry and become susceptible to wind erosion or other forms of resuspension.

This hypothetical conceptual model, if shown to be true, can result in tools that are immediately useful for minimizing exposure to the fungus. Landsat satellite imagery can be used to identify large highly degraded locations or ongoing and planned construction within the setting of endemic areas of the pathogen that together with wind data indicate high risk areas for *Coccidioides* exposure. Maps can be retrieved for past years as well, which allows the documentation of changes in risk of exposure to the pathogen due to changes in environmental conditions including human activities. Together with more information about the lifecycle of the fungus, these tools can be used to inform management decisions about certain activities. For example, it is possible that exposure to the fungus from travel on unpaved roads could be greatly reduced by adding a buffer between the travel portion of the road and the adjacent vegetation.

These ideas suggest a path forward for future applied research on this topic. Such research should focus on the length scales and time scales of influence that growth areas for the fungus exert along their border as well as obtaining a better understanding of the mechanism of direct physical suspension during land disturbance activities (i.e., how do infectious parts of the fungus get inhaled when a growth site is disturbed?). The following components are among those that ought to be included in future work:

1. Verify that the maximum exposure hazard is at the nexus of the somewhat ubiquitous (but highly dispersed) growth sites and activities that result in physical resuspension (e.g., earth moving activities) or Aeolian resuspension
2. Understand how far from the boundary between a vegetated growth area and an erodible area are infectious elements of the fungus available for resuspension and inhalation
3. Understand if there are specific periods or environmental conditions that are associated with the transfer of infectious elements from growth areas to erodible areas and once so transferred how long the material remains an infection hazard
4. Use large spatial datasets to define elevated conditions for exposure of military and civilian DoD personnel

These lines of inquiry likely require field measurements to test specific hypotheses and measure spatial and temporal extent of growth site influence as well as to verify the utility of large datasets. Ideally, such field work would include work within the boundaries of one or more DoD facilities in endemic areas (something which was not achievable for this pilot project). A controlled laboratory component where the growth cycle of *Coccidioides* is documented, especially in the context of the hypothesized mechanisms for exposure may also be warranted.

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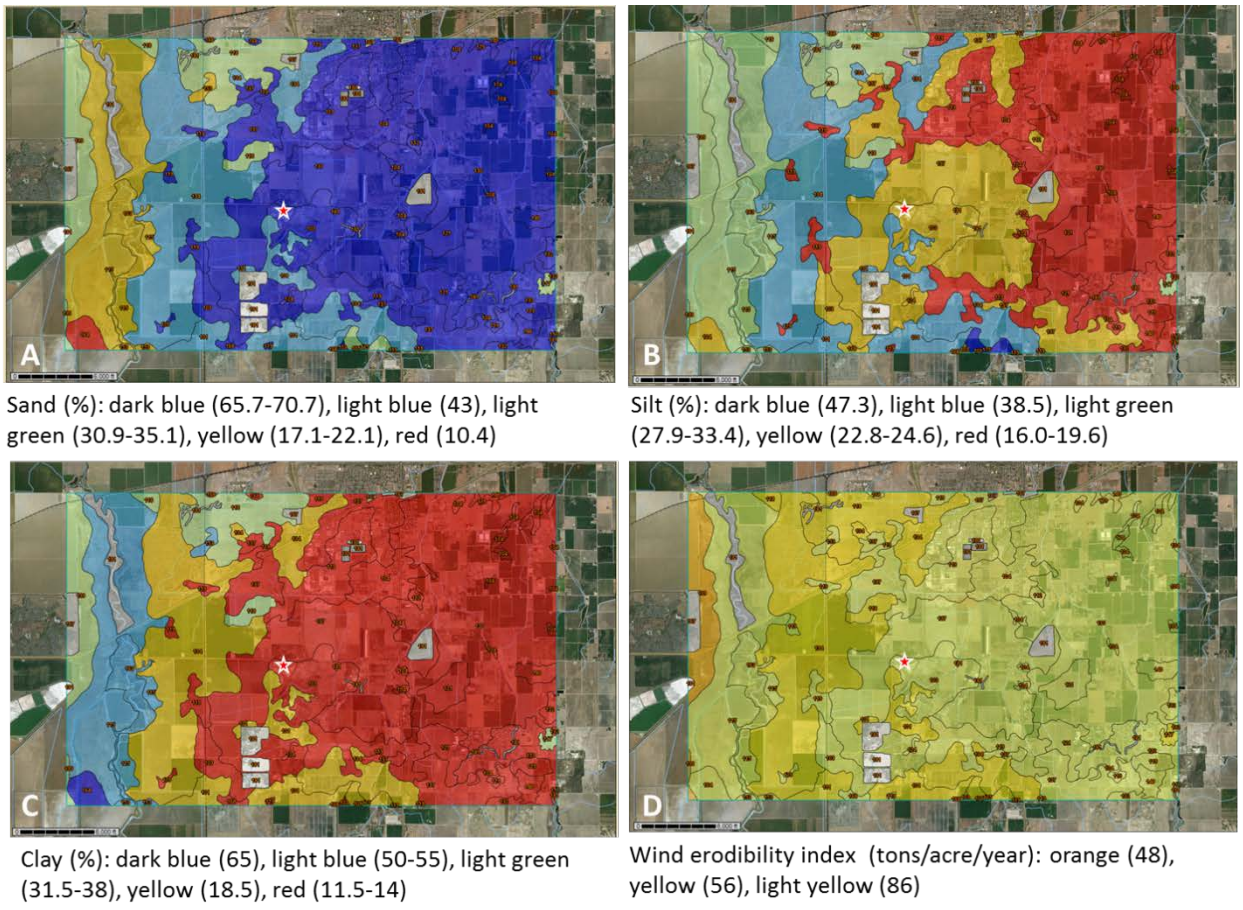
APPENDIX A



Sand (%): dark blue (65.7-70.7), light blue (43), light green (30.9-35.1), yellow (17.1-22.1), red (10.4)

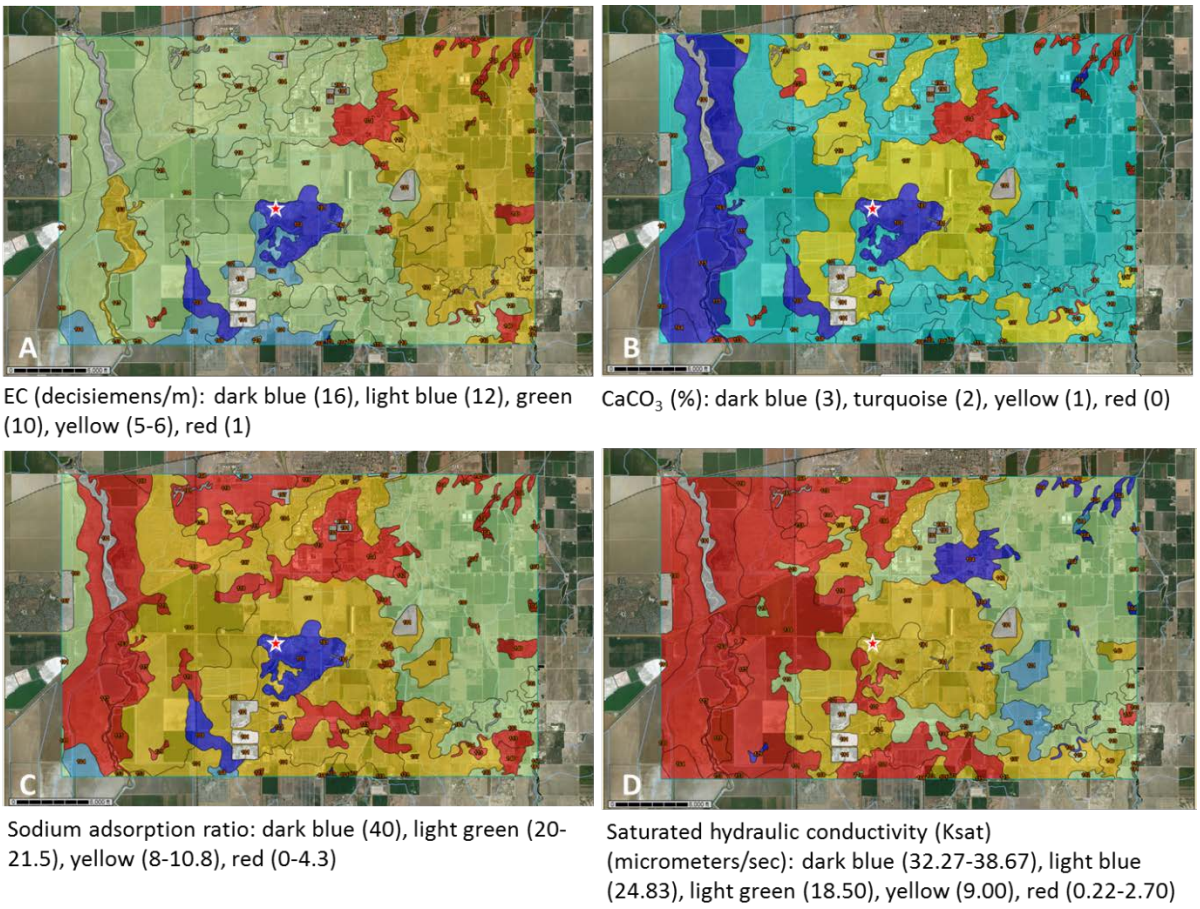
Silt (%): dark blue (47.3), light blue (38.5), light green (27.9-33.4), yellow (22.8-24.6), red (16.0-19.6)

Clay (%): dark blue (65), light blue (50-55), light green (31.5-38), yellow (18.5), red (11.5-14)

Wind erodibility index (tons/acre/year): orange (48), yellow (56), light yellow (86)

Figure 1. Grain Size Analysis (sand, silt and clay [%]) (A-C), As Well As Wind Erodibility (D) Indices for the Area Southeast of NAS Lemoore.

Coccidioides positive site J is indicated with a red star with white margin (data obtained from the USDA websoilsurvey database).



EC (decisiemens/m): dark blue (16), light blue (12), green (10), yellow (5-6), red (1)

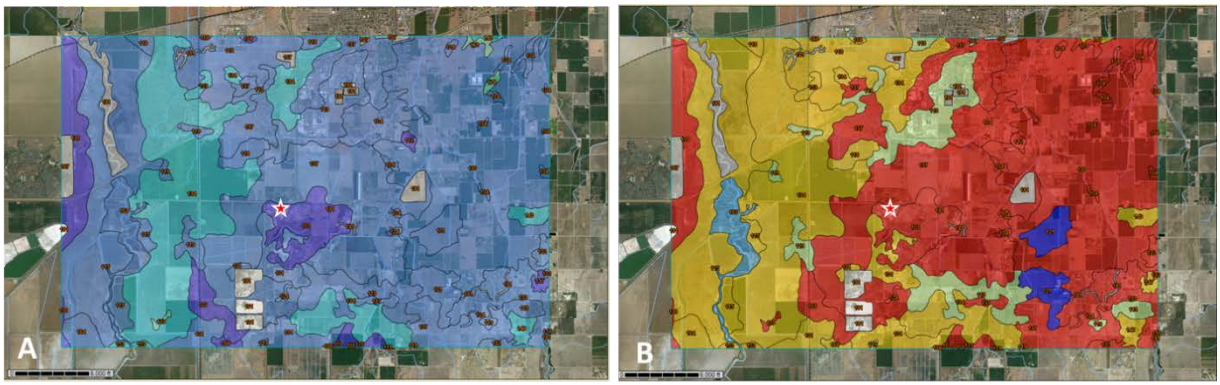
$CaCO_3$ (%): dark blue (3), turquoise (2), yellow (1), red (0)

Sodium adsorption ratio: dark blue (40), light green (20-21.5), yellow (8-10.8), red (0-4.3)

Saturated hydraulic conductivity (Ksat) (micrometers/sec): dark blue (32.27-38.67), light blue (24.83), light green (18.50), yellow (9.00), red (0.22-2.70)

Figure 2. Electrical Conductivity (A), $CaCO_3$ Content (B), Sodium Adsorption Ratio (C), and Saturated Hydraulic Conductivity (D) for Various Soil Types Southeast of NAS Lemoore.

Coccidioides positive site J is indicated with a red star with white rim (data obtained from the USDA websoilsurvey database).

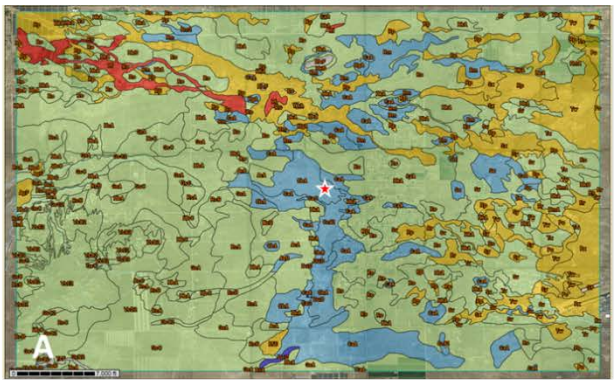


pH: purple (8.5), blue (7.9-8.20), turquoise (7.5-7.8), green (7.3)

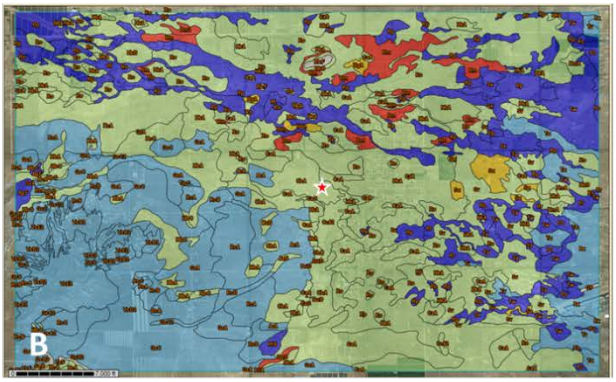
Organic matter (%): dark blue (3), light blue (2.5), light green (2.13), yellow (1.33-1.5), red (0.75-1.27)

Figure 3. pH (A) and Percent of Organic Matter (B) for Various Soil Types Southeast of NAS Lemoore.

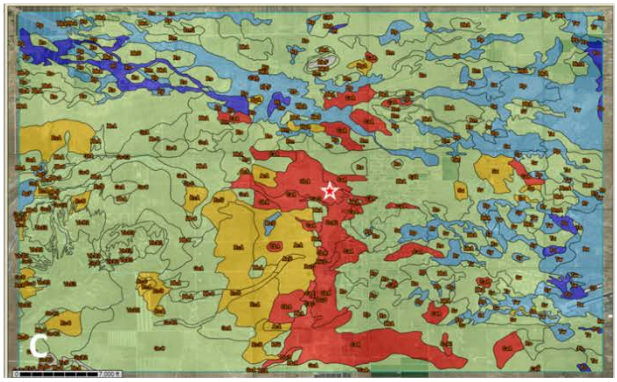
Coccidioides positive site J is indicated with a red star with white rim (data obtained from the USDA websoilsurvey database).



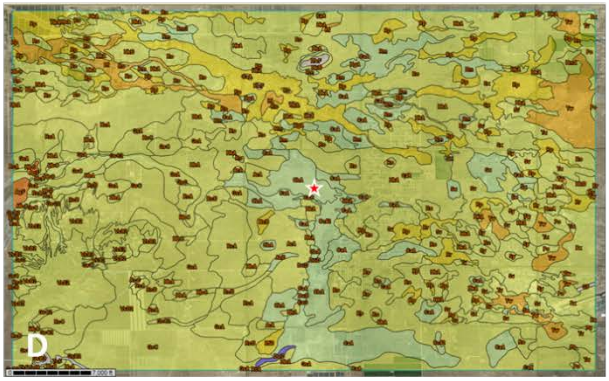
Sand (%): light blue (79.2-98.7), light green (60.6-70.5), yellow (42.1-52.1), red (18.1)



Silt (%): dark blue (35.5-50.9), light blue (19.2-24.9), light green (16.2-19.2), yellow (9.4), red (0-0.9)



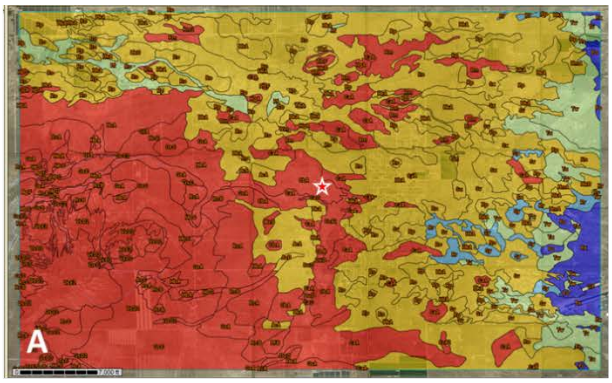
Clay (%): dark blue (31), light blue (17.5-23), light green (11-15), yellow (7.5-8.5), red (0.5-6)



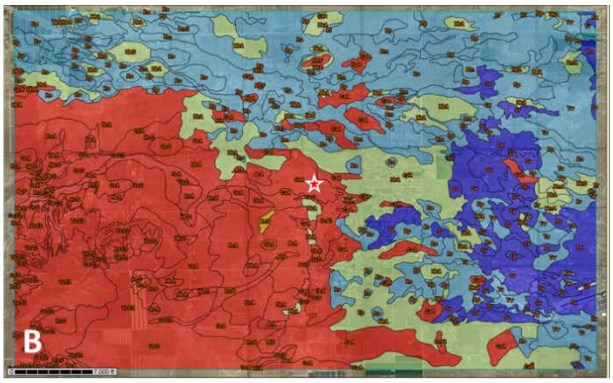
Wind erodibility index (tons/acre/year): dark blue (250), light yellow (86), yellow (56), orange (48)

Figure 4. Grain Size Analysis (sand, silt and clay [%]) (A-C), As Well As Wind Erodibility (D) Indices for the Area West of Antelope Acres.

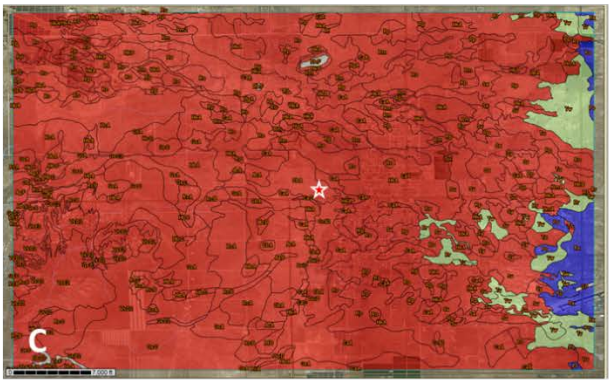
Coccidioides positive site 6 is indicated with a red star with white margin (data obtained from the USDA websoilsurvey database).



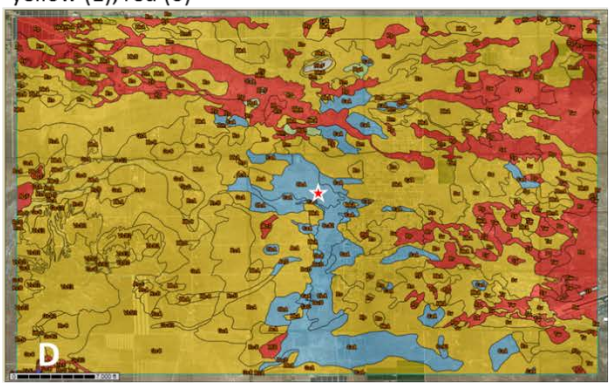
EC (decisiemens/m): dark blue (14), light blue (6-10), green (5.3), yellow (0.7-1.7), red (0-0.5)



$CaCO_3$ (%): dark blue (8), light blue (3), light green (2), yellow (1), red (0)



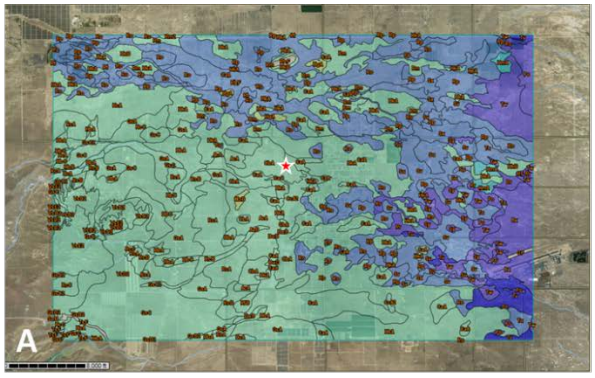
Sodium adsorption ratio: dark blue (11.5), light green (3), red (0)



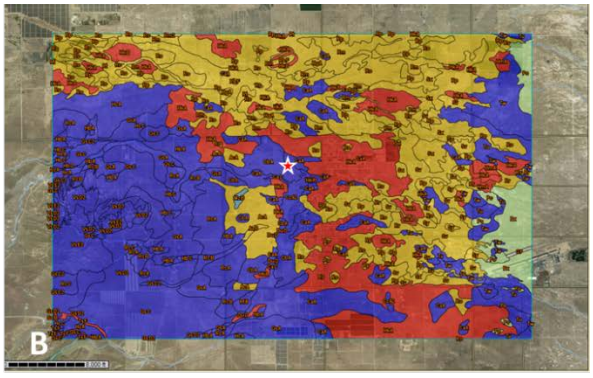
Saturated hydraulic conductivity (Ksat (micrometers/sec): light blue (92.00), light green (70.67), yellow (28.00), red (9-15.35)

Figure 5. Electrical Conductivity (A), $CaCO_3$ Content (B), Sodium Adsorption ratio (C), and Saturated Hydraulic conductivity (D) for Various Soil Types West of Antelope Acres.

Coccidioides positive site 6 is indicated with a red star with white rim (data obtained from the USDA websoilsurvey database).



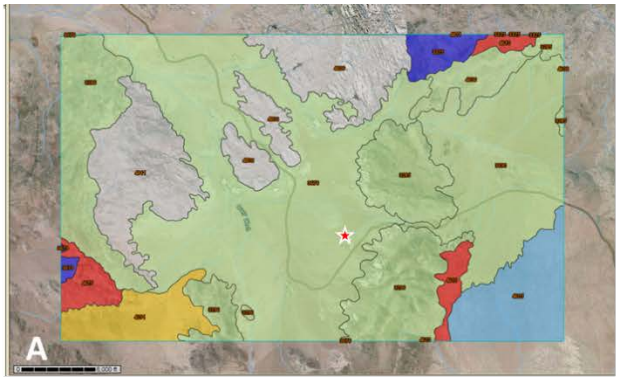
pH: dark blue (9.1), purple (8.5-9.0), blue (7.9-8.20), turquoise (6.5-7.8), green (6.2)



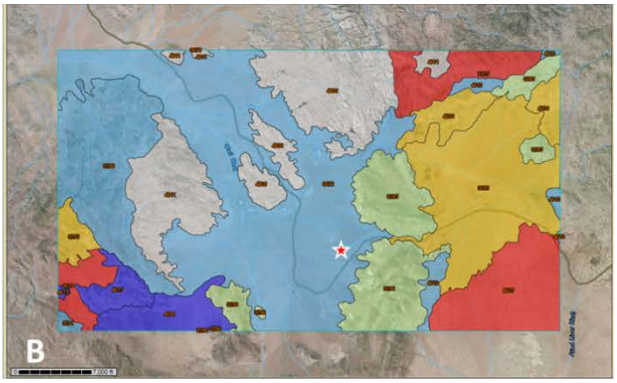
Organic matter (%): dark blue (0.75), light blue (0.56), light green (0.38), yellow (0.25), red (0.13)

Figure 6. pH (A) and Percent of Organic Matter (B) for Various Soil Types West of Antelope Acres.

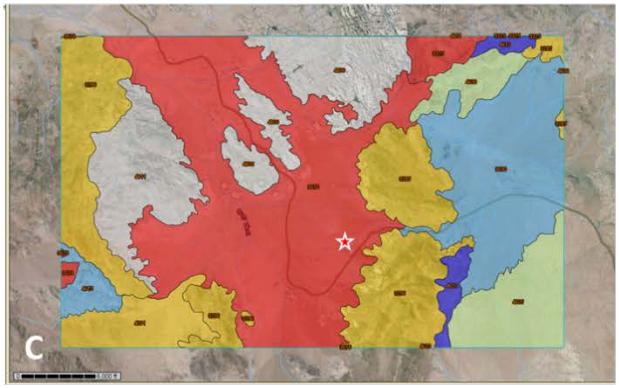
Coccidioides positive site 6 is indicated with a red star with white rim (data obtained from the USDA websoilsurvey database).



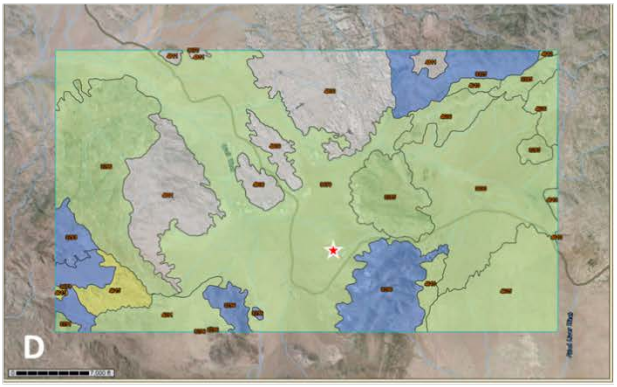
Sand (%): dark blue (93.2), light blue (85), light green (74.5-82), yellow (74.5), red (68.6-70.2)



Silt (%): dark blue (19.7-20.2), light blue (16-17), light green (12-13.3), yellow (10-10.6), red (4.3-7.2)



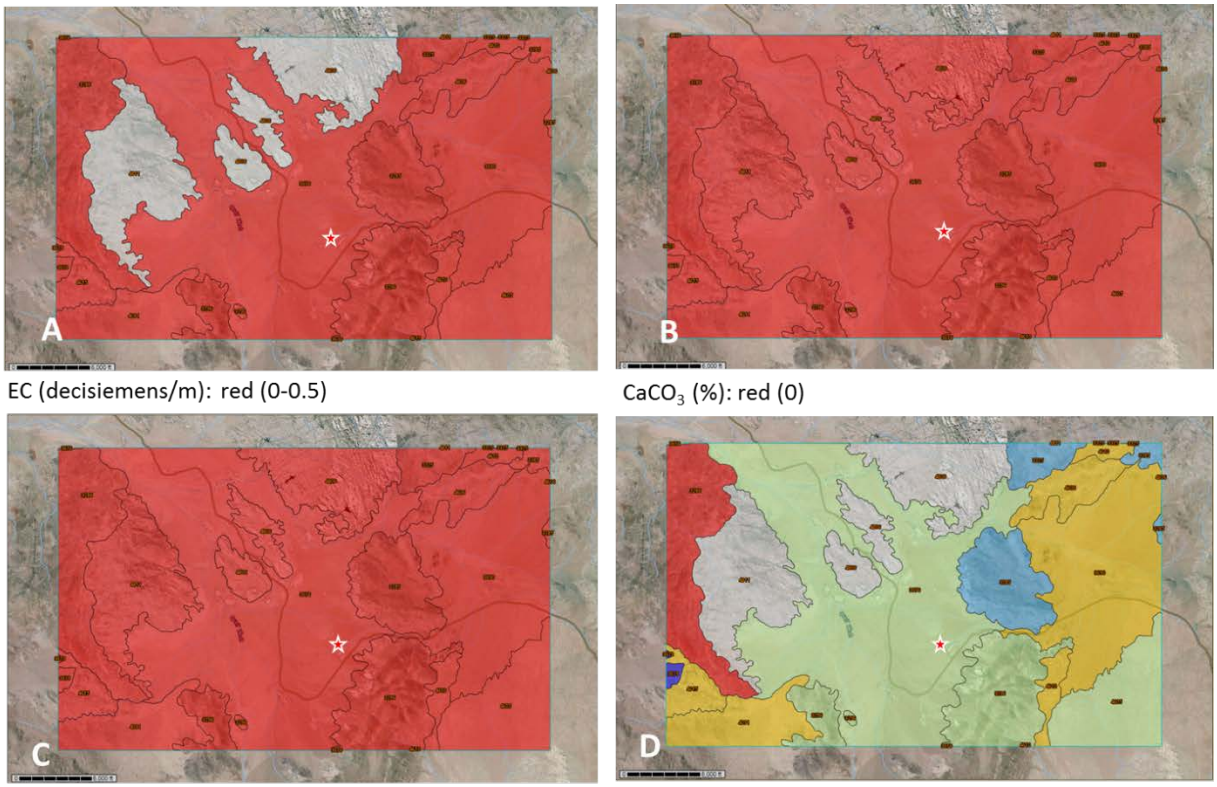
Clay (%): dark blue (14.7), light blue (9.6-9.7), light green (7.4-7.9), yellow (5.8-6), red (4)



Wind erodibility index (tons/acre/year): blue (220), light green (134), yellow (66)

Figure 7. Grain Size Analysis (sand, silt and clay [%]) (A-C), As Well As Wind Erodibility (D) Indices for the Area South of Twentynine Palms and MCAGCC.

Coccidioides positive site Hall of Horrors is indicated with a red star with white margin (data obtained from the USDA websoilsurvey database).



EC (decisiemens/m): red (0-0.5)

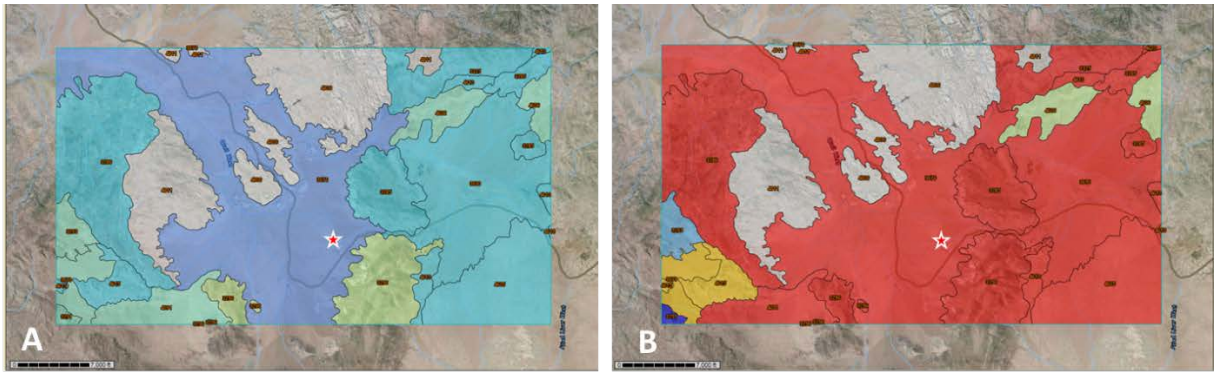
$CaCO_3$ (%): red (0)

Sodium adsorption ratio: dark blue (11.5), light green (3), red (0)

Saturated hydraulic conductivity (Ksat (micrometers/sec): dark blue (86.00), light blue (82.81), light green (68-70), yellow (25-57.25), red (37.75)

Figure 8. Electrical Conductivity (A), $CaCO_3$ Content (B), Sodium Adsorption Ratio (C), and Saturated Hydraulic Conductivity (D) for Various Soil Types in Joshua Tree National Park South of Twentynine Palms and MCAGCC.

Coccidioides positive site Hall of Horrors is indicated with a red star with white rim (data obtained from the USDA websoilsurvey database).



pH: turquoise (8.5-9.0), blue (8.2), light turquoise (7.2-7.3), green (6.5)

Organic matter (%): dark blue (0.50), light blue (0.33), light green (0.25), yellow (0.17-0.18), red (0.14-0.15)

Figure 9. pH (A) and Percent of Organic Matter (B) for Various Soil Types South of Twentynine Palms and MCAGCC of Antelope Acres.

Coccidioides positive site 6 is indicated with a red star with white rim (data obtained from the USDA websoilsurvey database).

Table A. Results of Diagnostic PCRs for Soil DNA Extracts from the Area Around NAS Lemoore for Samples Collected in January (n=56), May (n=32) and September 2017 (n=31).

Amplicons of correct size were sequenced and were 99-100 % related to GenBank nucleotide database entries C. immitis KY306695, C. posadasii KY399886 (VGITSf/r) and C. immitis EF186784, C. posadasii (KR109218 (EC3f/EC100r). Soil core samples were only collected in May and September (indicated in grey).

	Diagnostic PCRs	
Sample ID	**VG-ITS PCR (neg/pos)**	**EC PCR (neg/pos)**
LEMOORE 1/17 and 1/18/2017		
N26-1	strong pos	pos
N26-2	neg	neg
N26-3	very weak pos	neg
N26-4	neg	neg
N26-5	neg	neg
N26-6	very weak pos	neg
3_1	pos	neg
3_2	neg	neg
3_3	neg	neg
3_4	neg	neg
4_1	neg	neg
4_2	neg	neg
4_3	pos	pos
5_1	neg	neg
5_2	neg	neg
4_1	neg	neg
4_3	neg	neg
4_4	pos	neg
119-1	pos	neg
119_2	neg	neg
119-3	pos	neg
119-4	neg	neg
119-5	weak pos	neg
J-1	very weak pos	neg
J-2	very weak pos	neg
J-3	very weak pos	neg
J-4	strong pos	neg
115-1	neg	neg
115-2	very weak pos	neg
115-3	neg	neg
139-1-1	neg	neg
139-1-2	neg	neg
139-1-3	neg	neg
151-1	neg	neg
151-2	neg	neg
151-3	neg	neg
139-2-1	neg	neg
139-2-2	neg	neg
139-2-3	neg	neg
435-1	neg	neg
435-2	neg	neg
435-3	neg	neg
479-1-1	neg	neg

	Diagnostic PCRs	
Sample ID	**VG-ITS PCR (neg/pos)**	**EC PCR (neg/pos)**
479-1-2	neg	neg
479-1-3	neg	neg
477-1	neg	neg
477-2	neg	neg
477-3	neg	neg
462-1	neg	neg
462-2	neg	neg
482-1	neg	neg
482-2	neg	neg
475-1	neg	neg
475-2	neg	neg
479-2-1	neg	neg
479-2-2	neg	neg
LEMOORE 5/31/2017		
LM 1-1A	pos	pos
LM 1-1B	pos	neg
LM 1-1C	pos	pos
LM 1-1D	pos	pos
LM 1-2	pos	neg
LM 1-3	neg	neg
LM 2-1A	neg	neg
LM 2-1B	pos	neg
LM 2-1C	neg	neg
LM 2-1D	pos	neg
LM 2-1E	neg	neg
LM 2-1F	neg	neg
LM 2-2	neg	neg
LM 2-3	pos	neg
LM 2-4	neg	neg
LM 2-5	pos	pos
LM 3-1A	pos	pos
LM 3-1B	pos	pos
LM 3-1C	neg	neg
LM 3-1D	neg	neg
LM 3-1E	neg	neg
LM 3-1F	neg	neg
LM 3-2A	pos	neg
LM 3-2B	neg	neg
LM 3-2C	pos	neg
LM 3-2D	neg	neg
LM 3-2E	neg	neg
LM 3-2F	pos	neg
LM 4-1	neg	neg
LM 4-2	neg	neg
LM 4-3	pos	neg
core sample		
LEMOORE 9/15/2017		
N26-1	neg	neg
N26-2	neg	neg
N26-3	neg	neg

	Diagnostic PCRs	
Sample ID	**VG-ITS PCR (neg/pos)**	**EC PCR (neg/pos)**
N26-4	neg	neg
N26-5	neg	neg
Salt pit 1a	neg	neg
Salt pit 1b	neg	neg
Salt pit 1c	pos	pos
Salt pit 1d	neg	neg
site 3-1	neg	neg
site 3-2	neg	neg
site 3-2	neg	neg
site 3-3	neg	neg
site 3-4	neg	neg
site 3-5a	neg	neg
site 3-5b	neg	neg
site 3-5c	neg	neg
site 3-5d	neg	neg
site 4-1a	pos	pos
site 4-1b	neg	neg
site 4-1c	neg	neg
site 4-1d	neg	neg
site 4-1e	neg	neg
4-2	neg	neg
4-3	pos	neg
4-1	neg	neg
119-1	neg	neg
119-2	neg	neg
119-3	neg	neg
core sample		

Table B. Results of Diagnostic PCRs for Soil DNA Extracts from Antelope Acres, West of EAFB, for Samples Collected in February (n=36), May (n=40) and September (n=19) 2017.

Amplicons of correct size were sequenced and were 99-100 % related to GenBank nucleotide database entries C. immitis KY306695, C. posadasii KY399886 (VGITSf/r) and C. immitis EF186784, C. posadasii (KR109218 (EC3f/EC100r). Soil core samples were only collected in May and September (indicated in grey).

	Diagnostic PCRs	
sample ID	**VG-ITS PCR (neg/pos)**	**EC PCR (neg/pos)**
ANTELOPE ACRES 2/2/2017		
AA1-1	neg	neg
AA1-2	weak pos	neg
AA1-3	neg	neg
AA2-1	neg	neg
AA2-2	neg	neg
AA2-3	neg	neg
AA3-1	neg	neg
AA3-2	neg	neg
AA3-3	neg	neg
AA4-1	pos	neg

	Diagnostic PCRs	
sample ID	**VG-ITS PCR (neg/pos)**	**EC PCR (neg/pos)**
AA4-2	neg	neg
AA4-3	neg	neg
AA5-1	weak pos	weak pos
AA5-2	pos	pos
AA5-3	pos	pos
AA6-1	pos	pos
AA6-2	pos	pos
AA6-3	pos	pos
AA7-1	pos	pos
AA7-2	pos	pos
AA7-3	pos	pos
AA8-1	neg	neg
AA8-2	neg	neg
AA8-3	neg	neg
AA9-1	neg	neg
AA9-2	neg	neg
AA9-3	neg	neg
AA10-1	neg	neg
AA10-2	neg	neg
AA10-3	neg	neg
AA11-1	neg	neg
AA11-2	neg	neg
AA11-3	neg	neg
AA12-1	neg	neg
AA12-2	neg	neg
AA12-3	neg	neg
ANTELOPE ACRES 5/20/2017		
AA 2-1a	neg	neg
AA 2-1b	neg	neg
AA 2-1c	neg	neg
AA 2-1d	neg	neg
AA 2-1e	neg	neg
AA 2-1f	neg	neg
AA 2-2	neg	neg
AA 2-2a	neg	neg
AA 2-2b	neg	neg
AA 2-2c	neg	neg
AA 2-2d	neg	neg
AA 2-2e	neg	neg
AA 2-2f	neg	neg
AA 3-1	neg	neg
AA 3-2	neg	neg
AA 3-3	neg	neg
AA 5-1a	neg	neg
AA 5-1b	neg	neg
AA 5-1c	neg	neg
AA 5-1d	neg	neg
AA 5-1e	neg	neg
AA 5-1f	neg	neg
AA 5-2	pos	pos
AA 5-3	neg	neg

	Diagnostic PCRs	
sample ID	**VG-ITS PCR (neg/pos)**	**EC PCR (neg/pos)**
AA 6-1	neg	neg
AA 6-2	neg	neg
AA 6-3	neg	neg
AA 7-1a	pos	pos
AA 7-1b	neg	neg
AA 7-1c	neg	neg
AA 7-1d	neg	neg
AA 7-1e	neg	neg
AA 7-1f	neg	neg
AA 7-2a	neg	neg
AA 7-2b	pos	pos
AA 7-2c	neg	neg
AA 7-2d	neg	neg
AA 7-2e	neg	neg
AA 7-2f	neg	neg
ANTELOPE ACRES 9/12/2017		
AA 7-1a	neg	neg
AA 7-1b	neg	neg
AA 7-1c	neg	neg
AA 7-1d	neg	neg
AA 7-2	neg	neg
AA 7-3	neg	neg
AA 7-4	neg	neg
AA 7-5	neg	neg
AA 7-6	neg	neg
AA 7-7	weak pos	neg
AA 5-1a	neg	neg
AA 5-1b	neg	neg
AA 5-1c	neg	neg
AA 5-1d	neg	neg
AA 5-1e	neg	neg
AA 5-2	neg	neg
AA 5-3	neg	neg
AA 5-4	neg	neg
AA 5-5	neg	neg
core sample		

Table C. Results of Diagnostic PCRs for Soil DNA Extracts from Twentynine Palms and Joshua Tree NP, South of MCAGCC for Samples Collected in January (n=42), June (n=60) and October (n=75) 2017.

Amplicons of correct size were sequenced and were 98-100 % related to GenBank nucleotide database entries C. posadasii KY399886, C. immitis KY306695, (VGITSf/r) and C. posadasii (KR109218 (EC3f/EC100r). Soil core samples were only collected in June and October (indicated in grey).

	Diagnostic PCRs	
sample ID	**VG-ITS PCR (neg/pos)**	**EC PCR (neg/pos)**
29 PALMS 1/28/2017		
Twentynine Palms		
29p-1-1	strong pos	strong pos
29p-1-2	weak pos	pos
29p-1-3	neg	pos
29p-2-1	neg	neg
29p-2-2	strong pos	strong pos
29p-2-3	strong pos	strong pos
29p-3-1	neg	pos
29p-3-2	weak pos	pos
29p-3-3	neg	pos
29p-4-1	neg	pos
29p-4-2	pos	pos
29p-4-3	very faint pos	pos
29p-5-1	weak pos	neg
29p-5-2	neg	pos
29p-5-3	pos	pos
29p-6-1	neg	neg
29p-6-2	pos	pos
29p-6-3	weak pos	pos
29p-7-1	pos	pos
29p-7-2	weak pos	pos
29p-7-3	weak pos	pos
29p-8-1	neg	pos
29p-8-2	pos	pos
29p-8-3	neg	pos
29p-8-4	pos	pos
29p-9-1	strong pos	strong pos
29p-9-2	neg	neg
29p-9-3	strong pos	strong pos
29p-9-4	pos	pos
29p-10-1	strong pos	neg
29p-10-2	strong pos	strong pos
29p-10-3	neg	neg
Joshua Tree NP		
SR-1	strong pos	neg
SR-2	very faint pos	neg
HH-1	strong pos	neg
HH-2	neg	neg
HH-3	neg	pos
HH-4	strong pos	pos
HH-5	very faint pos	neg
HV-1	pos	pos

	Diagnostic PCRs	
sample ID	**VG-ITS PCR (neg/pos)**	**EC PCR (neg/pos)**
HV-2	strong pos	strong pos
HV-3	neg	neg
29 PALMS 6/10 and 11/2017		
Twentynine Palms		
29P 2-1a	pos	pos
29P 2-1b	neg	neg
29P 2-1c	neg	neg
29P 2-1d	pos	pos
29P 2-1e	neg	neg
29P 2-1f	neg	neg
29P 2-2	neg	neg
29P 2-3a	pos	pos
29P 2-3b	neg	neg
29P 2-3c	neg	neg
29P 2-3d	neg	neg
29P 2-3e	neg	neg
29P 2-3f	neg	neg
29P 5-1a	neg	weak pos
29P 5-1b	neg	neg
29P 5-1c	neg	neg
29P 5-1d	neg	neg
29P 5-1e	neg	neg
29P 5-1f	neg	neg
29P 5-2	neg	neg
29P 5-3a	neg	neg
29P 5-3b	neg	neg
29P 5-3c	neg	neg
29P 5-3d	neg	neg
29 P 6-1a	neg	neg
29P 6-1b	neg	neg
29P 6-1c	neg	neg
29P 6-1d	neg	neg
29P 6-1e	neg	neg
29P 6-1f	pos	pos
29P 6-2	neg	neg
29P 6-3	neg	pos
29P 7-1a	pos	pos
29P 7-1b	pos	pos
29P 7-1c	pos	pos
29P 7-1d	neg	neg
29P 7-1e	pos	pos
29P 7-1f	neg	neg
29P 7-2a	neg	neg
29P 7-2b	neg	pos
29P 7-2c	pos	pos
29P 7-2d	neg	neg
29P 7-2e	neg	neg
29P 7-2f	neg	neg
29P 7-3	pos	pos
29P 8-1a	pos	pos
29P 8-1b	neg	neg

	Diagnostic PCRs	
sample ID	**VG-ITS PCR (neg/pos)**	**EC PCR (neg/pos)**
29P 8-1c	neg	pos
29P 8-1d	neg	neg
29P 8-1e	neg	neg
29P 8-1f	neg	neg
29P 8-2	neg	neg
29P UT-1	neg	neg
29P UT-2	pos	pos
29P UT-3	neg	neg
Joshua Tree NP		
HH-1	neg	neg
HH-2	pos	pos
HH-3	pos	pos
HH-4	pos	pos
HH-5	neg	neg
core sample		
29 PALMS 10/13 and 14/ 2017		
Twentynine Palms		
29P 7-1a	neg	neg
29P 7-1b	neg	neg
29P 7-1c	weak pos	neg
29P 7-1d	neg	strong pos
29P 7-2	neg	neg
29P 7-3	neg	neg
29P 8-1a	pos	neg
29P 8-1b	neg	neg
29P 8-1c	neg	neg
29P 8-1d	neg	neg
29P 8-1e	neg	neg
29P 8-1f	neg	neg
29P 8-2	pos	neg
29P 8-3	neg	neg
29P 8-4	neg	neg
29P 9-1a	neg	pos
29P 9-1b	strong pos	pos
29P 9-1c	neg	neg
29P 9-1d	neg	neg
29P 9-1e	neg	neg
29P 9-1f	neg	neg
29P 9-2	neg	neg
29P 9-3	neg	neg
29P 10-1a	neg	pos
29P 10-1b	neg	pos
29P 10-1c	neg	neg
29P 10-1d	weak pos	neg
29P 10-1e	neg	neg
29P 10-1f	weak pos	neg
29P 10-2	pos	neg
29P 10-3	neg	neg
UT-1a	neg	neg
UT-1b	neg	neg
UT-1c	neg	neg

	Diagnostic PCRs	
sample ID	**VG-ITS PCR (neg/pos)**	**EC PCR (neg/pos)**
UT-1d	neg	neg
UT-1e	neg	neg
UT-1f	neg	neg
UT-2	neg	neg
UT-3	neg	neg
29P 6-1	neg	neg
29P 6-2	weak pos	pos
29P 6-3	neg	pos
29P 5-1	neg	neg
29P 5-2	neg	neg
29P 5-3	neg	neg
29P 4-1	neg	neg
29P 4-2	neg	neg
29P 4-3	weak pos	strong pos
29P 2-1	neg	pos
29P 2-2	neg	strong pos
29P 2-3	weak pos	pos
29P 1-1	neg	neg
29P 1-2	neg	neg
29P 1-3	weak pos	neg
29P 1-4	neg	neg
29P 1-5	neg	neg
29P 1-6	neg	neg
Joshua Tree NP		
HH-1a	neg	neg
HH-1b	neg	neg
HH-1c	neg	neg
HH-1d	neg	neg
HH-1e	neg	neg
HH-1f	neg	neg
HH-2	neg	neg
HH-3	neg	neg
HH-4a	neg	pos
HH-4b	neg	strong pos
HH-4c	neg	neg
HH-4d	pos	strong pos
HH-4e	neg	neg
HH-4f	neg	neg
HV-1	neg	neg
HV-2	neg	neg
HV-3	neg	neg
HV-4	neg	neg
core sample		

Table D. Detection of *Coccidioides* in Dust Samples Collected Around Antelope Acres, NAS Lemoore, and Twentynine Palms with Indication of Sample Type (PI-SWERL or TRAKER) Type of Diagnostic PCR and Coordinates of Sampling Locations.

The closest matches in the GenBank nucleotide database with percent similarity are indicated as well.

FilterID	Filter #	sample type and site	diagnostic PCR		closest match in GenBank			
			EC3f/EC100r	VGITSf/r	EC3f/EC100r	VGITSf/r	Lat	Lon
Antelope Acres (AA) 9/12/2017								
YUCFQ175	810	TRAKER	neg	neg				
YUCTQ171	hopper	site 7a	not enough sample				34.7467	-118.3069
YUCTQ171		site 7a-d	neg	neg			34.7467	-118.3069
YUCTQ175		site 5 a-c	neg	pos.		C. imm. KY306695, 100%, C. pos., KR109218, 100%	34.7393	-118.3038
dust on car		near site 8	neg	neg				
Lemoore 9/15/2017								
YUCTQ173	389	TRAKER, Lemoore,21st Str. North of Jackson	neg	neg				
YUCTQ177	521	TRAKER, Lemoore N26	neg	neg			36.377	-119.9325
YUCTQ177	hopper						36.377	-119.9325
YUCTQ176		PISWERL, site 1b (N26)					36.377	-119.9325
YUCTQ176		PISWERL, site 1c (N26)	neg	neg			36.377	-119.9324
YUCTQ176		PISWERL, site 1d (N26)					38.377	-119.9329
YUCTQ175		PISWERL, site 1a (N26)	neg	neg			36.372	-119.9322
YUCFQ174	947	PISWERL, saltpit 1					36.3717	-119.9317
YUCFQ174		PISWERL, saltpit 2	neg	neg			36.3717	-119.9317
YUCFQ174		PISWERL, saltpit 3					36.3717	-119.9317
YUCFQ174		PISWERL, saltpit 4					36.3801	-119.9214
YUCFQ177	848	PISWERL, site 4a	neg	neg			36.2547	-119.81
YUCFQ177	hopper	PISWERL, site 4b	neg	weak pos		not sequenced	36.2547	-119.81
YUCFQ173		PISWERL, site 3a	neg	neg			36.2558	-119.8345
YUCFQ173	292	PISWERL, site 3b					36.2558	-119.8345
no ID	hopper	PISWERL, site 3	not enough sample					
YUCFQ172	101	TRAKER, Lemoore 21st Str., to Bakersfield on-road sample, near intersection to Highway	neg	neg				
29 Palms 10/13/2017								
YUCTQ178	951	PISWERL, site 7a					34.1368	-116.0193
YUCTQ178	hopper	PISWERL, site 7	neg	neg			34.1368	-116.0193
YUCTQ178		PISWERL, site 7b					34.1368	-116.0193
YUCFQ182	hopper	PISWERL, site 8	neg	neg			34.1358	-116.003
YUCFQ182	943	PISWERL, site 8a	weak pos	neg	not sequenced		34.1358	-116.003
YUCFQ182		PISWERL, site 8b					34.1358	-116.003
YUCFQ178	105	PISWERL, site 9a	weak pos	neg	not sequenced		34.1361	-115.9717
YUCFQ178	hopper	PISWERL, site 9a					34.1361	-115.9717
YUCFQ181	555	PISWERL, site 10a					34.1343	-115.9116
YUCFQ181		PISWERL, site 10b	weak pos	neg	not sequenced		34.1343	-115.9116
YUCFQ181		PISWERL, site 10c					34.1343	-115.9116
YUCTQ172	77	PISWERL, site UTa	neg	neg			34.1957	-116.0364
YUCTQ172		PISWERL, site UTb					34.1957	-116.0364
YUCTQ179	124	PISWERL, site 6a	neg	neg			34.2592	-116.4407
YUCTQ179		PISWERL, site 6b					34.2592	-116.4407
YUCFQ179	360	PISWERL, site 5a					34.2657	-116.4459
YUCFQ179		PISWERL, site 5b	weak pos	neg	not sequenced		N/A	N/A
YUCFQ179		PISWERL, site 5c					34.2657	-116.4459
YUCTQ182	415	TRAKER, 29 Palms, near sites 7-10	pos	pos.	C. pos., AB232892, 98%, C. imm., AB232891, 98%,	C. pos, KY399886, 100%		

Table E. Summary of Results.

Comparison of all sites regarding landscape, land use, environmental parameters, presence of Coccidioides in soil and dust, as well as risk of exposure to the pathogen (EC=electrical conductivity, TDS=total dissolved salts, for details about soil chemical composition see graph showing results of ion chromatography, figures 10-13, Appendix).

	soil analyses (averages or ranges)		detection of *Coccidioides*		environment		
location/site description	soil chemical analyses	soil physical analyses	soil (wet season)	dust (dry season)	landform	vegetation	risk of *Coccidioides* exposure
Lemoore: dominated by agricultural activities, ~6.4 in rainfall, soil types: clay loam, sandy loam, fine sandy loam, clay many saline-sodic, alkaline	**pH:** 7.8-8.1, **EC:** 1 mS/m^3 (winter) - 13 mS/m^3 (summer), **TDS:** 0.4-6.1 g/L (winter-summer), **$CaCO_3$:** (3.51%), high levels of sulfate	sand (39.7%), silt (53.7%), clay (6.6%)	3.6% (confirmed with both primer pairs)	site 4 (PISWERL)	alluvial fans	mostly agricultural fields and orchards, non native trees, grasses, meadows, few semi-natural areas with salt bushes and iodine bushes	low
Antelope Acres (west of EAFB): western Mojave Desert, declining agricultural activities, former ranchland, increase in soil disturbance due to renewable energy construction, eroded soils, windy, ~7.4 in rainfall, soil types: coarse sandy loam, loamy sand	**pH:** 7.1-7.2, **EC:** <1 mS/m^3 (winter-summer), **TDS:** <0.1 g/L (winter-summer), **$CaCO_3$:** (0.05%) high levels of potassium	sand (62.3%), silt (30.6%), clay (3.6%)	25% (confirmed with both primer pairs)	site 5 (PISWERL)	alluvial fans, dry lakes	invasive grasses, rabbit brush, salt bushes, native and non-native herbs and wildflowers, eroded former farmland and non-vegetated areas (solar ranches)	high, incresing
Twentynine Palms and Joshua Tree NP: Southern Mojave Desert, semi-disturbed desert, windy, no agriculture, scattered settlements, ~4.4 in rainfall, outcrop associations, loamy sand, coarse loamy sand	**pH:** 7.5-8.0, **EC:** <1 mS/m^3 (winter-summer), **TDS:** <0.5 g/L (winter-summer), **$CaCO_3$:** (29 Palms: 0.65%, JT NP: 0.11%) high levels of potassium and calcium	29 Palms: sand (69.4%), silt (27.3%), clay (3.3%) JT NP: sand (55.9%), silt (35.7%), clay (3.1%)	52.4% (confirmed with both primer pairs)	sites 5, 8, 10 (PISWERL), sites 7-10 TRAKER	fan aprons, alluvial fans	desert shrub, creosote bushes, salt bushes, Joshua Trees, cacti, desert oak and pines, few invasive grasses, native and non-native herbs and wildflowers	high

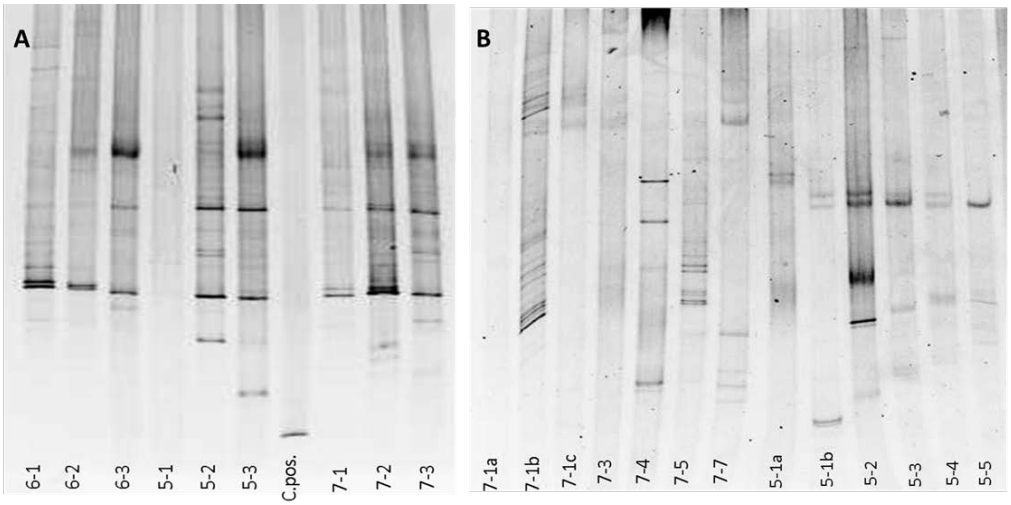


Figure 10. DGGE Profiles of Fungal Species in Soils Collected at Antelope Acres (west of EAFB) in the Winter (A) and in the Late Summer (B) of 2017.

The overall diversity is low in most samples as indicated by few distinct bands. In some samples fungal species are absent or not very abundant (no visible DGGE bands). The DGGE profiles also reveal a shift in diversity with depth and within habitats at the same location, as well as changes in time. None of the DGGE profiles indicate the presence of Coccidioides. Therefore, we can conclude that it is not a dominant member of the fungal community in these soil samples.

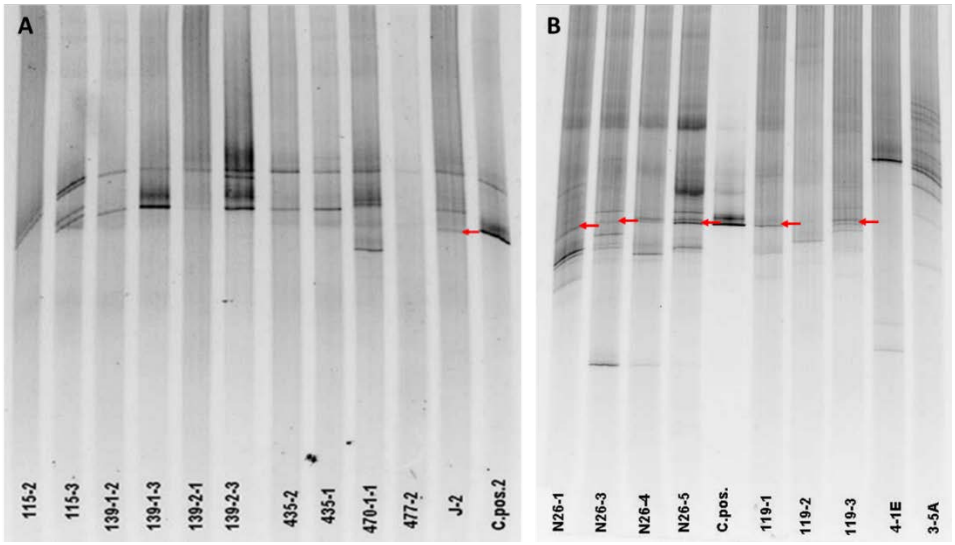


Figure 11. DGGE Profiles for Several Soil Samples Collected Around NAS Lemoore (winter 2017).

The red arrow indicates a band that is located in the same melting area of the gel as the positive control C. posadasii (very far right of figure 20A). A: DNA extracts from agricultural fields, except for sample J2. This sample was confirmed positive for the pathogen via diagnostic PCR. A dust sample collected from site J via PISWERL was also positive. B: DNA extracts from non-agricultural sites, a dirt road and adjacent ditch (N26), a dirt road and meadow (119), saline-alkaline environments (3-5A [surface sample] and 4-1E [30 cm depth sample]). The overall fungal diversity was low especially for samples from agricultural fields, showing between 2 and 4 distinct bands only. Near surface samples from more natural sites show a richer diversity as indicated by more than 5 bands in the DGGE profiles.

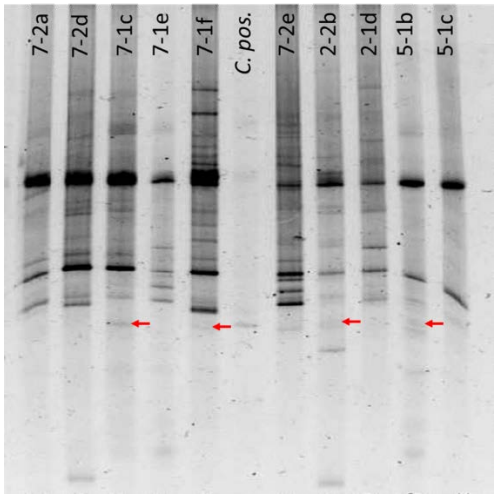


Figure 12. DGGE Profiles for Soil Samples Collected West of Antelope Acres (spring 2017).

The red arrows indicate bands that are located in the same melting area of the gel as the positive control C. posadasii (in the center). However, this could not be confirmed by diagnostic PCR. Sampling sites 5 and 7 were indicated as Coccidioides positive sites in the winter, and several core samples were collected in the following spring.

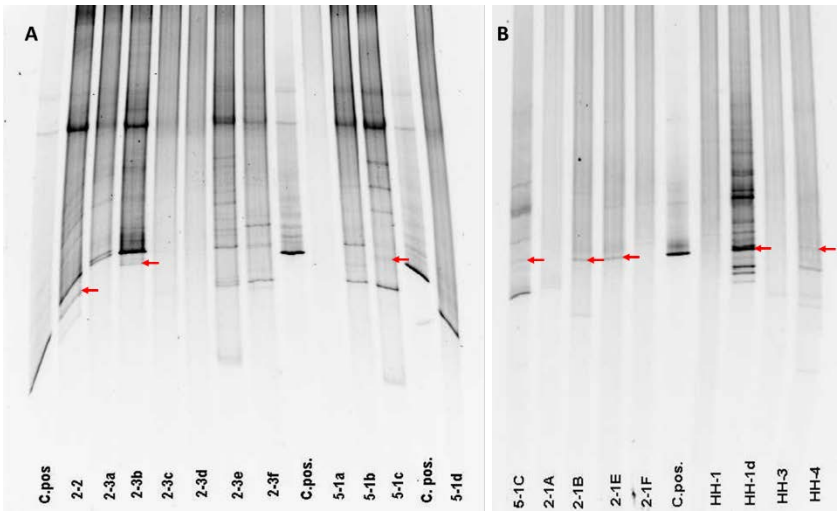


Figure 13 A and B. DGGE Profiles for Several Soil Samples Collected Around Twentynine Palms (spring 2017).

Multiple profiles show a band in the melting area that could represent Coccidioides (indicated with a red arrow). However, only sample HH-4 was indicated positive via diagnostic PCR. Note that the fungal diversity changes with depth for core samples 2-3a-2-3f and 5-1a-5-1d (A). The diversity was generally low with the exception of a sample from Joshua Tree NP (HH-1d, 18-20 cm depth).

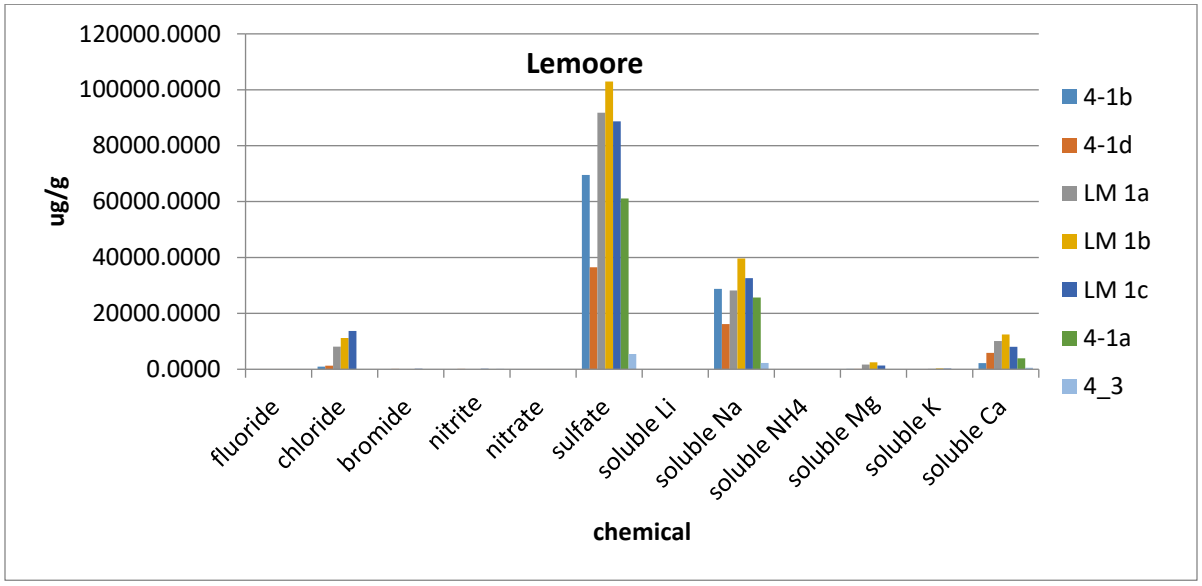


Figure 14. Mineral Composition of Soils Collected Around NAS Lemoore as Determined Via Ion Chromatography.

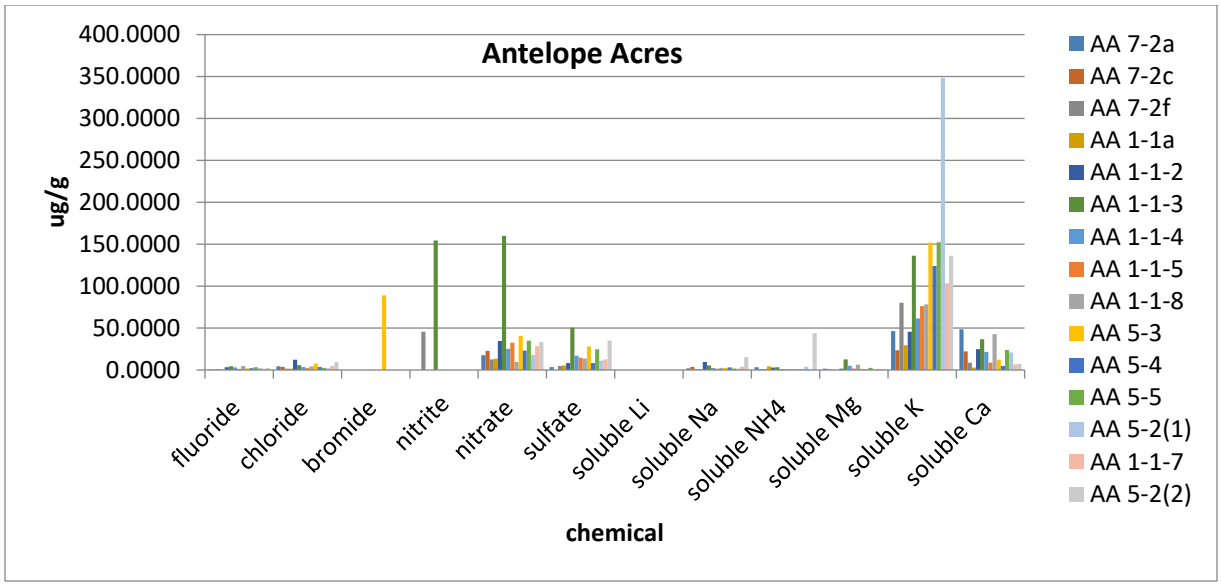


Figure 15. Mineral Composition of Soils Collected Around Antelope Acres, West of EAFT, as Determined Via Ion Chromatography.

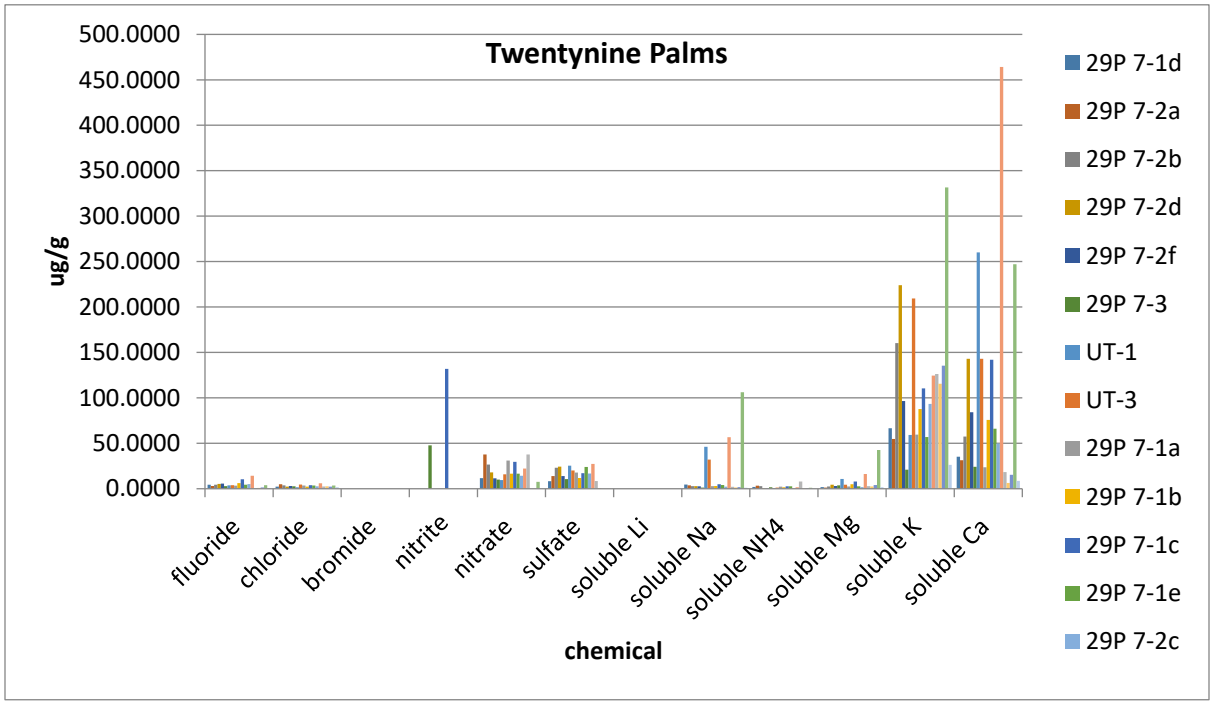


Figure 16. Mineral Composition of Soils Collected Around Twentynine Palms and Within Joshua Tree NP, as Determined Via Ion Chromatography.

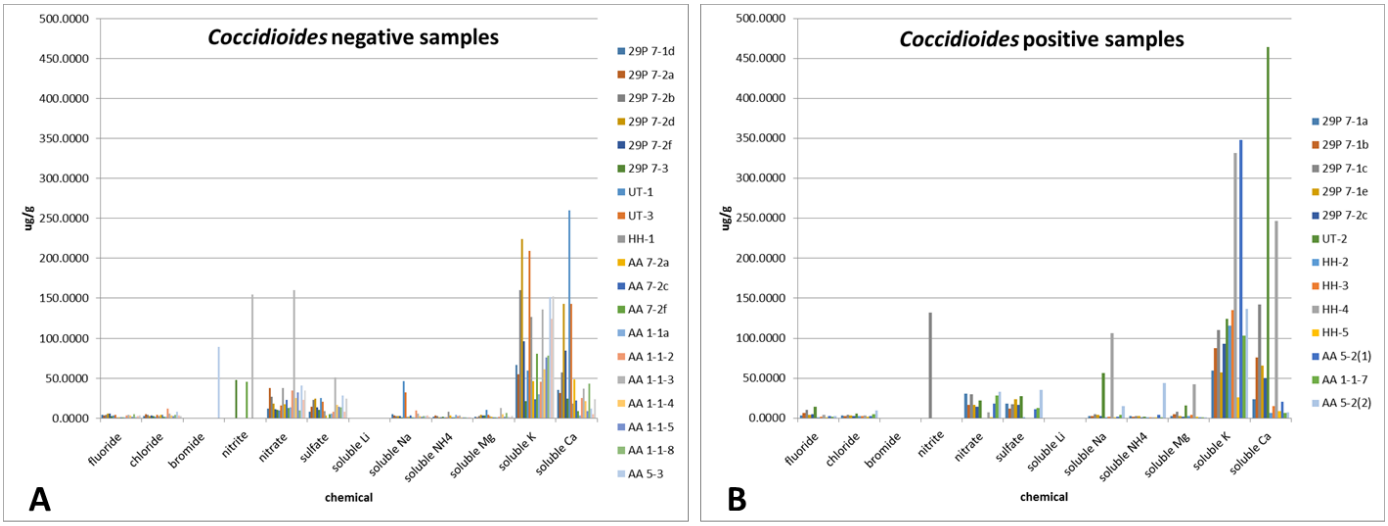


Figure 17. Mineral Composition of Soils that Tested Negative for *Coccidioides* (n=23) Compared to Soils that Tested Positive for the Pathogen (n=18) (only Antelope Acres, Twentynine Palms and Joshua Tree NP).

Lemoore samples were excluded because of their significantly higher amounts of some chemicals.