

# Large-Scale Land Development, Fugitive Dust, and Increased Coccidioidomycosis Incidence in the Antelope Valley of California, 1999–2014

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Abstract Ongoing large-scale land development for renewable energy projects in the Antelope Valley, located in the Western Mojave Desert, has been blamed for increased fugitive dust emissions and coccidioidomycosis incidence among the general public in recent years. Soil samples were collected at six sites that were destined for solar farm construction and were analyzed for the presence of the soil-borne fungal pathogen *Coccidioides immitis* which is endemic to many areas of central and southern California. We used a modified culture-independent nested PCR approach to identify the pathogen in all soil samples and also compared the sampling sites in regard to soil physical and chemical parameters,

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degree of disturbance, and vegetation. Our results indicated the presence of C. immitis at four of the six sites, predominantly in non-disturbed soils of the Pond-Oban complex, which are characterized by an elevated pH and salt bush communities, but also in grassland characterized by different soil parameters and covered with native and non-native annuals. Overall, we were able to detect the pathogen in 40% of the soil samples (n = 42). Incidence of coccidioidomycosis in the Antelope Valley was positively correlated with land use and particulate matter in the air (PM10) (Pearson correlation coefficient >0.5). With the predicted population growth and ongoing large-scale disturbance of soil in the Antelope Valley in coming years, incidence of coccidioidomycosis will likely further increase if policy makers and land developers continue to ignore the risk of grading land without implementing long-term dust mitigation plans in Environmental Impact Reports.

**Keywords** Fugitive dust · Renewable energy · Coccidioidomycosis · Mojave Desert · Soil

## Introduction

Large-scale land development in the Antelope Valley, located in northern Los Angeles County in California, provides new residences for expanding populations, facilities for businesses, fields for agriculture, and more recently provided opportunities for renewable energy production. However, arid and semiarid areas in the Southwestern US may require better care in managing soil disturbance from such projects because of greater risk of fugitive dust emissions and coccidioidomycosis, caused by the soil-born fungal pathogen Coccidioides spp. Fugitive dust is the suspension of particulate matter in the air by wind or human activities usually indicated as particulate matter up to 10 µm (PM10). The particulate matter is primarily soil but can contain crystalline silica, asbestos fibers, heavy metals, and airborne spores and conidia from microorganisms. Fugitive dust in general can cause breathing difficulties, low acute and chronic respiratory illnesses, increased risk of death from aggravated heart or lung disease [2, 12, 25, 27], increased risk of traffic accidents from poor road visibility [4], and reduced agricultural crop yield and desertification [75]. Fugitive dust emissions observed in the Antelope Valley frequently exceed California standards of 50  $\mu$ g/m<sup>3</sup> for PM10 (24 h averages) and 30  $\mu$ g/m<sup>3</sup> (annual arithmetic mean), respectively, which are stricter than federal standards (see http://www.arb.ca. gov/research/aaqs/caaqs/caaqs.htm for current California Ambient Air Quality Standards [CAAQS]). The increase in air pollution with coarse particulate matter (PM10) has raised the concern of public health officials and the general public [59], because of increased incidence of coccidioidomycosis among residents of the Antelope Valley (County of Los Angeles Department of Public Health, Annual Morbidity and Specials Studies Reports 2000-2014). Incidence of coccidioidomycosis in the Antelope Valley increased about 13-fold between 2000 and 2014 (supplementary figure S1). Strong Santa Ana winds can deliver dust from the desert to the LA Basin and deliver conidia of the pathogen to an area that is thought to be non-endemic for the pathogen [58].

The Antelope Valley is located in the Western Mojave Desert within the endemic zone of *Coccidioides* spp. which is comprised of certain areas in Arizona, California, Nevada, New Mexico, Texas, Utah, Washington, and Central and South America (see map in [55]). Fugitive dust that carries arthroconidia of *Coccidioides immitis* or *C. posadasii* can cause coccidioidomycosis in humans and animals primarily through inhalation of these dormant forms of the pathogen. Coccidioidomycosis primarily affects the pulmonary system in people and animals [16, 23],

but dissemination of the disease to other organs can occur [28, 57]. Although about 60% of infected people develop mild to no symptoms, the other 40% experience weeks to months of debilitating disease that can include fatigue, shortness of breath, cough, fever, night sweats, loss of appetite or weight, chest pain, headache, body aches, skin rash, and pneumonia [63]. Less than 5% of these patients develop disseminated coccidioidomycosis, which increases the risk of lifelong complications and death [23, 43, 71]. Despite considerable efforts, no vaccine to protect humans from coccidioidomycosis currently exists [74].

The issue of fugitive dust carrying *Coccidioides* spp. arthroconidia is important not just for workers involved in land development projects, but also for residents of nearby communities, residents of newly built neighborhoods, and visitors working, studying, or travelling through the area. Furthermore, strong winds can transport conidia far distances, sometimes hundreds of miles, which can cause disease in humans and animals in non-endemic areas [21, 34, 35, 56].

The Antelope Valley of California provides an opportunity to examine how changes in the environment due to large-scale land development effect incidence of coccidioidomycosis in humans. Consisting of over 1800 mi<sup>2</sup> of fertile lands, the Antelope Valley is located approximately 2500 ft above sea level and is part of the "Lower Sonoran Lifezone" [53], sometimes referred to as the "High Desert," a common name for a subregion located mostly in northwestern San Bernardino County, northeastern Los Angeles County, and far eastern Kern County in areas above 2000 ft in altitude [39, 77]. The valley experiences an annual precipitation of 6-9 inches per year, a mean annual high temperature of 98 °F in the summer and 59 °F in the winter, with temperatures commonly above 100 °F in July and August [51]. Mountains along the Southern and Western border of the Mojave Desert block most of the moisture-bearing westerly winds from the coast, limiting precipitation and air humidity, and strong prevailing winds can result in severe dust storms [62].

The Antelope Valley has the greatest potential for land development in Los Angeles County, and its land use increased notably between 2001 and 2011 (Fig. 1). Guevara et al. [33] showed that disturbance of soil during the "housing boom" that peaked between 2004 and 2005 was positively correlated with a spike in coccidioidomycosis incidence at the same time. In recent years, the Antelope Valley has become the focus of renewable energy projects to provide solarand wind-generated energy for Southern California [11, 38]. Solar farms constructed by multiple companies will ultimately cover more than 30,000 acres in the valley (e.g. [17, 19, 31], for an overview of all planned renewable energy projects). Overall, the DRECP affects approximately 22,858,000 acres of semiarid and arid soils in the counties of Los Angeles, Kern, Inyo, San Bernardino, Riverside, Imperial, and San Diego.

## Purpose and Scope

This project aimed to determine whether C. *immitis* is established in soils destined for photovoltaic system construction in the Antelope Valley, characterize the ecologic features of C. immitis positive sites, and correlate field findings with existing epidemiologic, geologic, and geographic data. Soil samples collected at six photovoltaic system sites either completed or destined for construction by 2014 or 2015 (Bureau of Land Management [BLM], CA, DRECP) were tested for the presence of Coccidioides spp. with a cultureindependent polymerase chain reaction (PCR)-based approach. The sampled sites included non-disturbed locations covered with natural vegetation, predominantly Atriplex polycarpa; disturbed grassland with native and non-native annuals; fallow agricultural fields; and land impacted by sheep grazing. With this study, we hope to raise awareness of an increasing environmental health hazard that has been neglected in the past. Policy makers and others involved with largescale land development projects could use the results from this study to implement better dust control approaches with more stringent requirements to reduce fugitive dust emissions and incidence of coccidioidomycosis and other dust-related illnesses among construction workers and the general public.

## **Materials and Methods**

#### Soil Sampling Area

All soil sampling sites were located in the Antelope Valley subsection of the Western Mojave Desert in northern Los Angeles County west of the city of Lancaster and south of the rural town Antelope Acres (Fig. 2). The Antelope Valley watershed is a large topographic depression with no hydrologic outlet to the ocean. The runoff into the basin from surrounding creeks is conveyed via broad ephemeral washes toward several dry lakes. Two large dry basins, or playas, the Rosamond and Roger's dry lake beds (Kern County) form dominant natural landscape features within the Antelope Valley and are located east of the sampling area.

Ecological Landscape Characterization of Sampling Sites

Soil samples were collected at six sites destined for solar panel construction. These locations included site 1: North Lancaster Silverado Project, site 2: West Antelope Silverado Project, site 3: American Silverado Project, site 4A and B: Antelope Silverado Project, site 5: Silver Sun Silverado Project, and site 6: Lancaster WAD Project. Soil parameter information for all sites was obtained from the United States Department of Agriculture (USDA) websoilsurvey database. Coordinates of all sampling spots were documented, and the appearance of soils, as well as the vegetation cover (plant species and degree of coverage and disturbance), was documented. Plant species were identified using the Jepson Desert Manual [5] and other literature [49, 54]. Rodent activity was observed at all sites in form of pellets, burrows or both. Soil samples were collected from soil types that were dominant in the locations destined for solar panel constructions and were collected from 5 to 7 cm depth. The pH of all soil samples was analyzed as well (two replicates). Pictures of all sampling sites can be seen in Fig. 3. Detailed site descriptions can be found in supplementary table S1.

## Soil Samples Collection

Thirty-one samples were collected at six sites on May 14 and 16 2014. Three to six individual soil samples (~25 g) were collected aseptically at several individual sampling spots at each of the six locations, using a small garden shovel and 50-ml Falcon tubes. After evaluation of all results from the 2014 sampling set, additional 11 soil samples were collected in May 2016 from site 6 only. All samples were transported to the laboratory on ice to prevent changes in the microbial communities and were stored at -20 °C before being



Fig. 1 Overview of land use in the Antelope Valley in 2001 (a) compared to 2011 with indication of renewable energy projects (b). The six sampling sites investigated in this study are indicated as *yellow circles*. (Color figure online)

processed the following week. The sampling sites were documented photographically, coordinates were determined, and vegetation cover and visual appearance of all soils in regard to disturbance, erosion, rodent activity, soil moisture, and soil color was described.

#### DNA Extraction and PCR

Soil samples were first mixed thoroughly by vortexing until homogenized. Prior to DNA extraction using the Powersoil DNA extraction kit (MoBio, Carlsbad, CA), 0.25 g of each soil sample was transferred into buffercontaining MoBio Powerbead tubes and incubated at 70 °C for 30 min, followed by an incubation step with 100 µl proteinase K (10 mg/ml) at 56 °C for additional 30 min [79] to enhance lysis of microbial spores and conidia. DNA extraction was performed according to the manufacturer's protocol (MoBio, Carlsbad, CA) using a MoBio vortex adapter for the beadbeating process. Two replicates were analyzed for each sample. The amount of DNA was quantified using the Qubit<sup>TM</sup> 3.0 Fluorometer (Invitrogen Life Technologies, Carlsbad, CA).

To determine the presence of C. immitis in all soil samples, a nested PCR approach based on the method published by Baptista-Rosas et al. [7] was used with modifications. A nested PCR can be superior to a onestep PCR method in that it excludes non-target DNA, therefore reducing possibilities of non-specific amplification. As the final diagnostic PCR step, we used 3 different primer pairs: (1) We replaced the originally suggested diagnostic primer pair with the ITSC1Af/ ITSC2r primer pair (~220 bp, ITS 2 region) published by Greene et al. [32], which we found superior in specificity for Coccidioides spp. than the diagnostic primer pair used in Baptista-Rosas et al. [7] (data not shown), which was originally published by Binnicker et al. [9]. (2) We also used the EC3f/EC100r diagnostic primer set [36, 37] to detect C. immitis, which amplifies a ~500-bp amplicon, large enough to distinguish the 2 species within the genus Coccidioides and which covers both ITS regions of the ribosomal gene. (3) We also used the diagnostic primer pair ITS1Cf/ ITS1Cr which amplifies a ~130 bp region of the ITS1 region of the ribosomal gene, published by Vargas-Gastélum et al. [76]. Overall, three sets of primers were used for each nested PCR approach. Aliquots of all PCR amplicons were analyzed using 2% (wt/vol) agarose gel electrophoresis to determine the correct size of the amplicons using a PCR marker (Promega G3161) (Promega Madison, WI) and ethidium bromide staining (0.5 mg/l). The first primer combination NSA3/NLC2 targets the ribosomal gene (18S and 5.8S DNA and both ITS regions) of all fungi and results in a ~1,100-bp amplicon. Amplicons from the NSA3/ NLC2 combination were then used as a template in a nested PCR approach using primer combination NSI1/ NLB4 which results in a ~910-bp fragment targeting a fragment of the ribosomal gene of Basidiomycetes and Ascomycetes only (see [7] for details). The final PCR step was the diagnostic PCR using a 1:25 dilution of the amplicons obtained with primer pair NSI1/NLB4 as a template and one of the diagnostic primer sets mentioned earlier in a final PCR. All PCR reactions were performed in duplicate, and the PCR cycling conditions as described in the original protocols were used (see Table 1 for details). PCR reactions contained 12.5 µl of GoTaq Green Mastermix (Promega, Madison, WI), 1.5 µl of each primer (10 pmol/µl), 2 µl of DNA extract or 1.5 µl of the product of a previous PCR reaction for the nested PCRs, as well as sterile water to a final volume of 25 µl. Negative control reactions, which contained all reactants with the exception of template DNA, were also included in all amplifications. These controls were carried through the entire nested PCR process along with the environmental products. Leftover PCR amplicons obtained via diagnostic PCR of approximately correct size (~220, ~500, ~130 bp) were subsequently treated with exoSAP-IT (Affymetrix, Santa Clara, CA), sequenced at the Center for Bioinformatics at the University of Florida, and subsequently compared to entries in the GenBank nucleotide database available at the National Center of Biotechnology Information (NCBI) (http://blast.ncbi. nlm.nih.gov/Blast.cgi) [1]. The sequencing step was necessary because occasionally false-positive amplicons were obtained.

#### Analysis of pH

Soil pH was determined on a 1:1 (w/v) soil/water mixture composed of 5 g of soil and 5 mL deionized



water. Samples were stirred before and after an equilibration period of 1 h and were then measured with an Oakton-510 bench-top pH meter (Oakton

Instruments, Vernon Hills, IL) after calibration to pH buffers 4, 7 and 10. Two replicates were performed for each soil sample and the average was determined.

◄ Fig. 2 a Aerial view of the Western Mojave Desert with indication of our sampling area (red rectangle) in the Antelope Valley, west of the city of Lancaster (Los Angeles County). b Aerial photo of the Antelope Valley as of April 2015 (landsat 8). Red numbers indicate all sampling sites (site 1 North Lancaster Silverado project, site 2 West Antelope Silverado Project, site 3 American Silverado Project, site 4A and B Antelope Silverado Project, site 5 Silver Sun Silverado Project, site 6 Lancaster WAD project). The red circles indicate areas where photovoltaic stations were constructed between 2009 and 2015. Construction sites outside these circles were not completed when this study was undertaken. The city of Lancaster is indicated in the lower right corner of the photo, south of the Rosamond dry lake bed. Also indicated are the Antelope Valley Poppy Reserve, the Arthur B. Ripley Desert Woodland State Park and the Mira Loma Detention Center. The settlement Antelope Acres is situated between the construction sites west of Foxfield Airport. (Color figure online)

## Results

## DNA Extraction and PCR

DNA of high quality was successfully extracted from all samples as confirmed by 2% agarose gel electrophoresis and subsequent ethidium bromide staining which resulted in distinct bands of non-sheared DNA. The amount of DNA extracted from 0.25 g of soil varied between soil samples and ranged between 29.2 and 9780 ng/ml. Site 6 had the smallest amount of DNA extracted (29.2–2420 ng/ml), whereas DNA extractions from samples collected at site 4 resulted in the highest amount of extracted DNA (3840– 9780 ng/ml) (Table 2).

The nested PCR approach to detect Coccidioides spp. confirmed DNA of fungal origin in all soil samples and also confirmed DNA of Ascomycetes and/or Basidiomycetes in 90% of the samples. An example of nested PCR results including all three individual PCR steps with all diagnostic primer pairs is shown in Fig. 4 for a subset of samples. Table 2 summarizes the results of all PCRs and includes the closest matches in the GenBank nucleotide database for all sequenced amplicons. After comparing all sequences to entries in the Genbank nucleotide database, 17 soil samples (40.48%) were found positive for the pathogen with PCR amplicons of 99 or 100% similarity to a C. immitis entry in the GenBank nucleotide database (6.7, collected in 2016, showed a faint band of correct size with primer pair ITS1Cf/ITS1Cr which could not be confirmed by sequencing). Two additional soil samples resulted in amplicons that were 89% related to C. posadasii (sites 4B3 and 5.4 collected in 2014). Most of the falsepositive PCR products were related to fungi in the order Capnodiales (Cladosporium spp.). In some occasions multiple species contributed to an amplicon, resulting in a "noisy" sequence that could not be identified. PCR products obtained with diagnostic primer pair EC3f/EC100r resulted more often in falsepositive results than PCR products obtained with primer pair ITSC1Af/ITSC2r. Diagnostic primer pair ITS1Cf/ITS1Cr was the most specific of all three primer pairs tested, resulting in no false-positive amplicons. This primer pair was also the most sensitive one, because it detected the pathogen in 23.81% of the samples (28.57% if the two unconfirmed samples are considered as well). Primer pair EC3f/EC100r detected the pathogen in 11.9% of the samples, while primer pair ITSC1Af/ITSC2r detected C. immitis in 19.05% of the samples. Samples 6.2 collected in 2016 was the only sample that tested positive for the pathogen with all three diagnostic primer pairs. Five samples collected in 2014 and one sample collected in 2016 were indicated positive with two out of the three diagnostic primer pairs. Individual sampling spots where the pathogen was detected are shown in supplementary figure S2. Examples of high-quality sequences obtained with all 3 diagnostic primer pairs were deposited in the GenBank nucleotide database available at the National Center for Bioinformatics and Information (NCBI) (Accession No. KY306689-KY306699).

Characterization of Soil Samples

Variation in soil characteristics was observed for all sampling sites (USGS Soil Survey Antelope Valley, www.usdawebsoilsurveydatabase; Table 3; and supplementary figure S3). Soils in the sampling area varied in soil parent material, and in regard to chemical and physical parameters, as indicated by different USDA soil map units. Overall, the soil types that were the most common in our sampling area were characterized as Hesperia fine sandy loam (~10% of the sampling area), Greenfield sandy loam (~18%), Cajon loamy sand (~5.5%), Pond loam (5%), Rosamond fine sandy loam (~4%), Sunrise sandy loam (~6.5%); several others each covered <4% of the study area. Soils belonging to the Pond-Oban complex covered a large area of the valley around Rosamond and Roger's



◄ Fig. 3 Landscape overview of all sampling sites at the time of sampling (May 2014). a Site 1, a disturbed site with scattered native and non-native vegetation. b Site 2, grassland with native and non-native annuals. c Site 3, disturbed land with scattered native and non-native vegetation. Surrounding areas grew rabbit brush (*Ericameria nauseosa*) (as can be seen in the *background*). d Site 4A, a disturbed area with scattered native and non-native species. g and h Site 6, dominated by scattered salt bushes and occasional rabbit brush. Dried *Lastenia californica* can be seen in between the salt bushes (*Atriplex* spp.)

dry lake bed and comprised ~15% of the eastern study area where sampling site 6 was located. Soil pH generally increased with proximity to the Rosamond dry lake bed and ranged between pH 5 and 9.4. The pH varied considerably for subsamples from sites 2, 4A and 6, but were more uniform among samples from sites 1, 3, 4B and 5. Furthermore, average soil pH results observed in our laboratory differed from the averaged values indicated in the USDA websoilsurvey database. For example, fine sandy loam samples from sites 1 and 6 appeared less alkaline when analyzed in our laboratory. Samples positive for C. immitis also varied in pH, but the majority of the positive soil samples showed a pH higher than 7 and less DNA could be retrieved compared to samples with a lower pH (Fig. 5; supplementary figure S4). Soil parameters that were indicative of the presence of C. immitis in previous research in the Southern San Joaquin Valley (Kern County) [44, 45] predicted site 6 in the Antelope Valley as a potential growth site of the pathogen (Table 3). However, sites 2, 3 and 5 where the pathogen was detected as well were not indicated as potential growth sites based on soil parameters. Other soil types near our sampling sites, such as Rosamond loam and Tray loam, share some of the parameters that were indicative of the presence of the pathogen in the San Joaquin Valley, but these soils were not

**Table 1** Position of primers on ribosomal gene (A), all primer pairs used for nested PCR reactions with PCR amplification conditionsand references (B)



f Forward primer, r reverse primer

<sup>a</sup> All samples were subjected to an initial melting step of 94 or 95 °C for 10 min and a final extension step of 72 °C for 10 min

ITSC2r,	EC3f/EC10	0r and ITSICt/II								
Sample ID	NSA3/ NLC2	NSI1/NLB4	ITSC1Af/ITSC	2r	EC3f/EC100r		ITS1Cf/ITS1C	Ţ	DNA extract	ed
2014	All fungi	Ascomycetes/ Basidiomycetes	Coccidioides spp.	% Similarity to closest match in GenBank	Coccidioides spp.	% Similarity to closest match in GenBank	Coccidioides spp.	% Similarity to closest match in GenBank	(ng/ ml)	Hd
1.1	Positive	Positive	Negative		False positive	unc. <i>Capnodiales</i> , JF691038, 99%	Negative		2800	6.90
1.2	Positive	Positive	Negative		False positive	unc. <i>Capnodiales</i> , JF691038, 94%	Negative		2640	7.13
1.3	Positive	Positive	Negative		False positive	unc. <i>Capnodiales</i> , JF691038, 95%	Negative		1960	7.13
2A.1	Positive	Positive	Positive	Coccidioides immitis, HG380500, 100%	False positive	unc. Capnodiales, JF691038, 93%	Positive	Coccidioides immitis, KM679413, 100%	3320	5.95
2.2	Positive	Positive	Negative		False positive	unc. Capnodiales, JF691038, 94%	Negative		4360	7.28
2.3	Negative	Negative	Negative		Negative		Negative		7280	6.90
2.4	Positive	Positive	Negative		False positive	unc. Capnodiales, JF691038, 90%	Negative		1406	5.24
2.5	Positive	Positive	Negative		False positive	unc. <i>Capnodiales</i> , JF691038, 93%	Negative		1858	6.29
2.6	Positive	Positive	Positive	Coccidioides immitis, HG380500, 100 %	Negative		Positive	Coccidioides immitis, KM679413, 100%	4700	7.12
3.1	Positive	Positive	Positive	Coccidioides immitis, HG380500, 99%	False positive	unc. Capnodiales, JF691038, 95%	Positive	Coccidioides immitis, KM679413, 100%	3520	6.73
3.2	Positive	Negative	Negative		÷	Multiple sequences***	Negative		3480	6.76
3.3	Positive	Positive	Negative		ż	Multiple sequences	Negative		2500	6.03
3.4	Positive	Positive	False positive	unc. Eurotiales, HQ389458, 95%	False positive	unc. <i>Capnodiales</i> , JF691038, 99%	Negative		6620	6.79
3.5	Positive	Positive	Positive	Coccidioides immitis, KJ783449, 100%	Positive	Coccidioides immitis, KJ783449, 100%	Negative		4400	7.11
4A.1	Positive	Positive	False positive	unc. Eurotiales, HQ389458, 96%	False positive	unc. <i>Capnodiales</i> , JF691038, 94%	Negative		3840	6.81
4A.2	Positive	Positive	Smear**		False positive	unc. Capnodiales, JF691038, 96%	Negative		9780	6.50
4A.3	Positive	Positive	Negative		False positive	Ascochyta sp., KC959210, 85%	Negative		6980	5.59
4B.1	Positive	Negative	Smear		False positive	unc. Capnodiales, JF691038, 95%	Negative		4520	7.38
4B.2	Positive	Positive	False positive		False positive		Negative		5900	755

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Table 2	continued									
Sample ID	NSA3/ NLC2	NSI1/NLB4	ITSC1Af/ITSC	2r	EC3f/EC100r		ITS1Cf/ITS1Cr		DNA extract	ed
2014	All fungi	Ascomycetes/ Basidiomycetes	Coccidioides spp.	% Similarity to closest match in GenBank <i>Trichophyton terrestre</i> , LN714614, 96%	Coccidioides spp.	% Similarity to closest match in GenBank unc. <i>Capnodiales</i> , JF691038, 94%	Coccidioides spp.	% Similarity to closest match in GenBank	(ng/ ml)	Hq
4B.3	Positive	Positive	False positive	Coccidioides posadasii, KF386150, 89%	False positive	unc. <i>Capnodiales</i> , JF691038, 94%	Negative		8020	7.36
5.1	Positive	Negative	Negative		False positive	unc. <i>Capnodiales</i> , JF691038, 94%	Negative		7040	6.67
5.2	Positive	Positive	False positive	Cladosporium macrocarpum, KC311478, 95%	False positive	unc. Capnodiales, JF691038, 92%	Positive	Coccidioides immitis, KM679413, 100%	3760	6.44
5.4	Positive	Positive	False positive	no similarity found	False positive	Coccidioides posadasii, JX051631, 89%	Negative		8040	69.9
5.5	Positive	Positive	Negative		False positive	Multiple sequences	Negative		3280	6.75
5.6	Positive	Positive	Negative		False positive	unc. <i>Capnodiales</i> , JF691038, 93%	Negative		7120	6.50
6.1	Positive	Positive	?	multiple sequences	Positive	Coccidioides immitis, KJ783449, 99%	Negative		1796	7.44
6.2	Positive	Positive	Positive	Coccidioides immitis, KJ783449, 99%	Negative		Negative		1270	8.15
6.3	Positive	Positive	Positive	Coccidioides immitis, KJ783449, 99%	Negative		Negative		2420	7.04
6.4	Positive	Positive	Smear		Negative		Negative		2080	7.00
6.5	Positive	Positive	Positive	Coccidioides immitis, KJ783449, 99%	Positive	Coccidioides immitis, KJ783449, 99%	Negative		174	9.45
6.6 2016	Positive	Positive	Negative		Negative		Positive	Coccidioides immitis, KM679413, 100%	29.2	8.79
6.1	Positive	Positive	Negative		Positive	No signal (faint PCR product)	Positive	Coccidioides immitis, KM679413, 98%	120	8.12
6.2	Positive	Positive	Positive	Coccidioides immitis, KJ783449, 99%	Positive	Coccidioides immitis, KJ783449, 94%	Positive	Coccidioides immitis, KM679413, 100%	85.8	7.38
6.3	Positive	Positive	Negative		Positive	Coccidioides immitis, KJ783449, 99%	Positive	No signal, faint PCR product	53	6.91
6.4	Positive	Positive	Negative		False positive	Fungal endophyte, KT291114, 94%	Positive	Coccidioides immitis, KM679413, 100%	1014	7.94
6.5	Positive	Positive	Negative		Negative		Negative		324	8.25
6.6	Positive	Positive	Negative		Negative		Negative		55.6	9.53

Table 2	continued									
Sample ID	NSA3/ NLC2	NSI1/NLB4	ITSC1Af/ITSC	'2r	EC3f/EC100r		ITS1Cf/ITS1Cr		DNA extrac	ted
2014	All fungi	Ascomycetes/ Basidiomycetes	Coccidioides spp.	% Similarity to closest match in GenBank	Coccidioides spp.	% Similarity to closest match in GenBank	Coccidioides spp.	% Similarity to closest match in GenBank	(ng/ ml)	μd
6.7	Positive	Positive	Negative		False positive	Fungal endophyte, KT291114, 96%	Positive	No signal, faint PCR product	964	7.85
6.8	Positive	Positive	Negative		Negative		Negative		210	6.84
6.9	Positive	Positive	Negative		False positive	No similarity found	Negative		458	7.55
6.1	Positive	Positive	Negative		False positive	No similarity found	Positive	Coccidioides immitis, KM679413, 100%	244	9.21
6.11	Positive	Positive	Negative		Negative		Positive	Coccidioides immitis, KM679413, 100%	766	7.31
Positve s	amples and	l their clostest mat	tch in the GenB	ank database are presented	l in bold					

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investigated in this study (soils where the pathogen was detected are indicated as positive [bold]).

Environmental Parameters and Incidence of Coccidioidomycosis

Environmental parameters, such as fugitive dust emission (PM10), total annual precipitation (inches), and wind speed (gust max.), were obtained for the Mojave Air Basin for the years 2000-2015. In addition, we obtained land-use data (acres) [15] and coccidioidomycosis incidence data [14] for the same time period and area (Fig. 6). An increase in incidence of coccidioidomycosis over time can be seen with highest incidence in the Antelope Valley in 2005, 2011 and 2014, spiking shortly after years with increased soil disturbance due to the "housing boom" between 2003 and 2007 [33] and the renewable energy boom described in this study. Between 2005 and 2014, the number of approved permits for solar farms and wind parks increased with additional large-scale and small-scale projects pending permission. So far, more than 20,000 acres of land have been disturbed as of 2014 for renewable energy projects in the Antelope Valley and the surrounding foothills of the Tehachapi and San Bernardino Mountains [30, 31]. The acreage of field crops increased by 48% compared to the year 2000 and then steadily declined by 2014 reaching values close to those documented before 2008 (County of Los Angeles Crop and Livestock Report 2014). The correlation between incidence of coccidioidomycosis in the Antelope Valley and the amount of acres of land disturbed for renewable energy projects and amount of acres under agricultural management (field crops) between 2000 and 2014 was strong, as revealed by a correlation coefficient of  $r^2 = 0.623$  (Pearson product-moment correlation coefficient) and  $r^2 = 0.388$ . The correlation between PM10 (Mojave Air Basin) and disease incidence was at best weak with a Pearson coefficient of 0.283 and an  $r^2$  value of 0.0664 (see Fig. 6 for all correlation values). However, the correlation between PM10 and incidence of the disease was strong when only the years between 2009 and 2014 were considered (renewable energy boom), with a Pearson coefficient of 0.641 and an  $r^2$ value of 0.411. To investigate these relationships in more detail, a multiple regression analysis was conducted (Table 4). This analysis shows that neither PM10 nor levels of precipitation appear to have had a



**Fig. 4** Displayed are 2% agarose gels after ethidium bromide staining, showing examples of diagnostic PCR results for samples from some locations. Sequences from PCR amplicons circled in black were 99% related to a GenBank database entries of *C. immitis. White arrows* point toward amplicons of correct size including some that were revealed as false positives. **a** Results of

amplification with primer pair ITSC1Af/ITSC2r showing amplicons of ~220. **b** Results of amplification with primer pair EC3f/ EC100r showing amplicons of ~500 bp. **c** Results of amplification with diagnostic primer pair ITS1Cf/ITS1Cr showing positive results for samples 5.2 and 6.6 (2014). (*C.i. Coccidioides immitis* used as positive control; *NC* negative control)

significant relationship with coccidioidomycosis incidence (2000–2014). However, the total acres of land under the three land-use types considered (wind, solar and agricultural) did have a significant, positive relationship with coccidioidomycosis incidence (p < 0.01) over the same time period. We investigated the relationship with land use further by conducting a second multiple regression, removing the non-significant factors and disaggregating the three land-use types, to determine whether effects could be attributed to specific type of lands use. This analysis revealed no significant differences between the effects of the different land-use categories (Table 5).

#### Discussion

A correlation between soil disturbances due to largescale renewable energy construction projects, agricultural management practices and PM10 fugitive dust emission with increased incidence of coccidioidomycosis was clearly indicated by results of this study. The increasing incidence of coccidioidomycosis in the Antelope Valley of California, which has reached epidemic character, is concerning and shown in supplementary figure S1. The C. immitis positive sites detected in this study are located west of the cities of Lancaster and Palmdale and south of the community of Antelope Acres which are part of what is known as the Greater Antelope Valley Economic Alliance (GAVEA) which has experienced a population increase of 24% between 2000 and 2010 (US Census Bureau). It has been predicted that the population will continue to grow another ~46% by 2035, to 758,881

residents [31]. The predicted population growth will result in continued urbanization as of yet unknown proportions, but certainly of significant size. Therefore, it is expected that fugitive dust emissions from ongoing construction sites will continue or even increase. This environmental health hazard will put humans and animals at an increased risk for contracting coccidioidomycosis, especially if dust mitigation continues to be inefficient or absent. In addition to increased urbanization and renewable energy development in this area, an ongoing drought with decreasing precipitation and sinking ground water tables has been blamed for soil erosion and fugitive dust development in the Antelope Valley. The ongoing drought has also resulted in a significant reduction in farming activities over the last years, resulting in large areas of abandoned fields. For example, the farmed acreage of orchards decreased from 2013 to 2014 by 53.06%, and the farmed acreage for grapes decreased by 22.6% during the same time in the County [48].

It has been difficult in the past to determine a clear correlation between incidence of coccidioidomycosis and certain environmental parameters, because of combined immediate or delayed positive or negative effects on the growth of the pathogen in the soil. Previous work by Talamantes et al. [72] determined a weak correlation between precipitation and wind speed and coccidioidomycosis incidence in Kern County. Smith et al. [69] and Kirkland and Fierer [40] had already pointed out that a rainy winter can cause an increase in coccidioidomycosis incidence in the following dry season, especially after a prolonged drought that might have caused a shift in the microbial community toward spore and conidia formers, among **Table 3** Averaged soil physical and chemical parameters for dominant soil types found in our sampling area with indication of soil map unit symbols, as obtained from the USDA websoilsurvey database (pH was also analyzed at CSUB). (Color figure online)

Soil parameters (averaged data)			Sampling sites		
Sites	1*	1, 3*	2*, 4A*, 4B*, 5	2, 4, 5*	6*
Map unit name	Rosamond fine	Hesperia fine	Greenfield	Ramona coarse	Pond Oban
	Sandy loam	Sandy loam	Sandy loam	Sandy loam	complex
Landform	Alluvial fans	Alluvial fans	terraces, alluvial fans	terraces	Basin floors
Parent material	Alluvium derived	Alluvium derived	Alluvium derived	Alluvium derived	Alluvium derived
	from granite	from granite	from granite	from granite	from granite
Map unit symbols	Ro	HkA	GsA/GsC	RcB/RcC	Px
Physical parameters					
Surface texture	Fine sandy loam	Sandy loam	Sandy loam	Coarse sandy loam	Fine sandy loam
Clay (%)	18.8	13	11	7.5	25.7
Sand (%)	51.9	70.5	66	69.6	46.5
Silt (%)	29.3	16.5	23	22.9	27.8
Available water capacity (cm/cm)	0.14	0.13	0.13	0.1	0.08
Available water supply (0–25 cm)	3.45	2.5	3.25	2.5	2.04
Organic matter (%)	0.17	0.08	0.75	0.75	0.25
Water content (15 bar) (%)	12.1	8.1	7.4	6	15.8
Water content (1/3 bar) (%))	23.3	17.2	16.4	14.7	27.5
Sat. hydraulic conductivity (Ksat) (µm/s)	21.67	28	28	28	4.8
Chemical parameters					
pH (websoilsurvey database)	8	7.4	6.7	6.7	8.9
pH (determined at CSUB)	7.1 (site 1)	6.7 (site 3)	6.4 (site 2), 6.1/6.6 (site 4A/4B)	6.8	8
CaCO <sub>3</sub>	5	2	0	0	8
Cation exchange capacity (CEC7) (milliequivalents/100g)	10	7.5	7.5	7.5	15.8
Gypsum	0	0	0	0	0
Sodium adsorption ratio (SAR)	0	0	0	0	12.7
Electrical conductivity (EC) (decisiemens/m)	1.7	0.7	0	0	14
Wind erodibility index (tons per acre per year)	86	86	86	86	86
Detection of C. immitis	Negative	Positive	Positive	Positive	Positive

Soil parameters			Other dominant soils in the	sampling area		
Sites	1	3	3	6	2	4
Map unit name	Rosamond	Cajon	Tray sandy loam,	Tray	Hanford	Sunrise
	loam	loamy sand	Saline-alkali	Loam	Coarse sandy loam	sandy loam
Landform	Alluvial fans	Alluvial fans	Basin floors	Basin floors	Alluvial fans	basin floors
Parent material	Alluvium derived	Alluvium derived	Alluvium derived	Alluvium derived	Alluvium derived	alluvium derived
	from granite	from granite	from granite	from granite	from granite	from granite
Map unit symbols	Rp	CaA	Tv	Tw	HbC	Sv
Physical parameters						
Surface texture	Loam	Loamy sand	Sandy loam	Loam	Sandy loam	Sandy loam
Clay (%)	20.5	3.7	12.7	20	12.5	15
Sand (%)	34.7	83.1	65.8	42.1	68.2	65.9
Silt (%)	44.8	13.2	21.6	37.9	19.3	19.1
Available water capacity (cm/cm)	0.16	0.08	0.1	0.15	0.13	0.12
Available water supply (0–25 cm)	3.85	1.98	2.5	3.5	3.25	3
Organic matter (%)	0.17	0.57	0.58	0.75	0.58	0.25
Water content (15 bar) (%)	12.3	3.3	8.4	12.5	8.9	9.2
Water content (1/3 bar) (%))	27.7	10.9	17.7	27.1	17.8	18.3
Sat. hydraulic conductivity (Ksat) (µm/s)	9	92	21.7	9	28	28
Chemical parameters						
pH (websoilsurvey database)	8.2	7.2	9.1	9	6.7	7.9
pH (determined at CSUB)			Not determine	d		
CaCO <sub>3</sub>	5	1	3	3	0	8
Cation Exchange Capacity (CEC7) (milliequivalents/100g)	10	3	7.5	7.5	7.5	7.5
Gypsum	0	0	0	0	0	0
Sodium adsorption ratio (SAR)	0	0	3	3	0	0
Electrical conductivity (EC) (decisiemens/m)	1.3	0.2	5	5	0	1
Wind erodibility index (tons per acre per year)	56	134	86	48	86	86
Detection of C. immitis			Not investigate	ed		

Soil parameters that were indicative of the presence of the pathogen in the Southern San Joaquin valley [44, 45] are indicated in red. Additionally, results from our PCR-based approach to detect *C. immitis* are included (at some sampling sites, more than one soil type was detected; therefore, the soil type of the soil samples analyzed is indicated with an \*; soil types where the pathogen was detected are indicated with a red rectangle)



**Fig. 5 a** Correlation between soil pH and amount of extracted DNA. **b** The amount of extracted DNA from *C. immitis* positive soil samples was significantly lower than the amount of DNA extracted from *C. immitis* negative soil samples. **c** The pH of soils in which the pathogen was detected was higher than the pH of

soils that were negative for the pathogen. However, the difference was not significant (data were normally distributed based on the Shapiro-Wilkes test for normality of the residuals). (Color figure online)

them *Coccidioides* spp. In our study, we were able to clearly link land use and soil disturbance to valley fever incidence, but also found a positive correlation between PM10 and wind speed; however, the correlation was rather weak. The continued increase in coccidioidomycosis incidence in 2012 and 2013 when construction of new renewable energy projects slowed down was likely due to the long-term effect of large areas of graded soils, which continue to be a major source of fugitive dust emission in the Antelope Valley and beyond. In the past, California had been plagued with long-term and short-term droughts, for example the prolonged drought from 1985 to 1992 which resulted in increased fugitive dust emissions that reached a 24-h record PM10 concentration of 780  $\mu$ g/m<sup>3</sup> in downtown Lancaster in 1991 (Antelope Valley Air Quality Monitoring District).

We were able to detect the pathogen *C. immitis* predominantly in undisturbed alkaline soils of the Pond-Oban complex, located closest to the Rosamond dry lake bed, a location commonly referred to as "barren land" with different species of salt bushes, that indicate a saline and alkaline environment. Site 6 was the only sampling site that was suspected to harbor *C. immitis* based on averaged soil parameter information (USDA websoilsurvey database) that



**Fig. 6** Increase in soil disturbance over time in the Antelope Valley due to large-scale renewable energy project construction, changes in agricultural management correlated with incidence of coccidioidomycosis. Displayed are approved solar and wind projects (acres), agricultural fields under management (acres), as well as the number of issued building permits and completed buildings (housing developments) between 2000 and 2014. Included in the calculations (but not included in the graph) is also PM10 data (High National 24-Hour Average), precipitation

(inches), and wind-speed data (gust max.) for the city of Lancaster measured at Foxfield Airport (KWJF). Solar and wind farms were graphed one year after the permit approval date because the begin of construction generally began in the year after permits were issued (data sources: www.arb.ca.gov/adam, http://publichealth.lacounty.gov/acd/Publications.htm, http:// planning.lacounty.gov/assets/upl/project/energy\_projects.pdf, http://pcd.kerndsa.com/planning/renewable-energy, http:// lacfb.org/crop-reports-2/)

**Table 4** Results of the initial multiple regression model: the model was coccidioidomycosis incidence = PM10 + precipitation+acres of land use (summed across Solar projects, wind projects, and active agricultural use

	Estimate	SE	t	р
(Intercept)	4.90	3.06	1.61	0.13
PM10	$-6.14 \times 10^{-3}$	$1.06 \times 10^{-2}$	-0.580	0.57
Annual Precipitation (in)	1.34	1.08	1.24	0.24
Acres of land use	$4.04 \times 10^{-4}$	$1.20 \times 10^{-4}$	3.36	$0.0057^{**}$

Final model is: coccidiomycosis incidence =  $4.9 - 0.0062 \times PM10 + 1.33$  precipitation + 0.00040 land use.  $F_{3,12} = 4.8$ , p < 0.05, multiple  $r^2 = 0.54$ 

were indicative of the presence of the pathogen in the Southern San Joaquin Valley [44, 45]. The Southern San Joaquin Valley Desert is geologically somewhat related to the Western Mojave Desert where the Antelope Valley is located, but differs in elevation and climate [24]. Soils of both locations developed from quaternary alluvium and similar underlying parent material and have been described as alluvial fans or fan remnants and basin floors, with high concentrations of fine particulate matter that accumulated since the late Pleistocene and earlier. However, the pathogen was also detected in grassland from soils characterized as Greenfield sandy loam, Hesperia fine sandy loam, and Ramona fine sandy loam (sites 2, 3 and 5). The grassland appeared similar to a strong growth site of the pathogen, Sharktooth Hill near Oildale, east of

**Table 5** Results of the 2nd multiple regression model: themodel was coccidioidomycosis incidence = acres of solarprojects + acres of wind projects + acres active agriculturaluse

	Estimate	SE	t	р
(Intercept)	-6.02	15.8	-0.38	0.711
Solar	$8.86 \times 10^{-05}$	$1.72 \times 10^{-03}$	0.052	0.96
Wind	$5.16 \times 10^{-05}$	$7.34 \times 10^{-04}$	0.070	0.945
Crop	$1.78 \times 10^{-03}$	$1.66 \times 10^{-03}$	1.07	0.306
$\overline{F_{3,12}} = 2.3$	p > 0.1, multip	le $r^2 = 0.36$		

Bakersfield, not far away from a severely disturbed area, the Kern River oilfields, but the physical and chemical parameters of soils from Sharktooth Hill (high clay content) were more similar to those determined at site 6. Fossil diggers repeatedly became infected with C. immitis at Sharktooth Hill, where the presence of the pathogen has been confirmed repeatedly [20, 45, 64, 70]. Overall, soils from all C. immitis positive sites in the Antelope Valley and the Bakersfield area can be characterized as fine particulate sandy loam. The investigation of other soil types should be included in future studies to refine the set of environmental parameters that are indicative of the presence of the pathogen and to deepen our understanding of the ecology of C. immitis in California. The diversity of habitats that C. immitis can apparently grow in indicates that the pathogen is able to adapt to somewhat different soil environments or that different ecotypes of the pathogen exist which might explain its "spotty distribution" [6, 20]. Furthermore, it should be noted that site 6 where the pathogen was detected repeatedly had the lowest amounts of extracted DNA. A fungal species such as Coccidioides spp. which is missing some key enzymes needed to grow successfully as a saprophyte in soil might benefit from a low diversity of overall soil microbes that could include potential competitors and antagonists [65].

It has been difficult and expensive to detect *Coccidioides* spp. in soil and dust samples in the past [8, 22], but modern culture-independent molecular methods became available in recent years which allow for successful screening of environmental samples for the presence of *C. immitis* and *C. posadasii* [7, 36, 37, 42, 44–46, 66, 76]. However, soil analyses for the detection of soil-borne pathogens, such as *Coccidioides* spp., have not been included in Environmental Impact Reports (EIRs) for any construction

project planned in the Antelope Valley or in other endemic areas of the pathogen in the Southwestern US. The scarcity of experts who are familiar with the procedures to detect the pathogen in its natural environment, additional costs of soil analyses, and a general underestimation of the risk of otherwise healthy people of contracting coccidioidomycosis from dust exposure might explain this potentially risky situation.

Mitigation and regulatory efforts are likely to be inadequate if the current trends in development and associated fugitive dust emissions continue. During spring 2014, fugitive dust emissions in the Antelope Valley negatively impacted air quality to an extent never documented before, reaching values up to and above 1000  $\mu$ g/m<sup>3</sup>, which reminded people of the Great Dust Bowl of the 1930's in Oklahoma [47], or the extreme dust storms documented in Owens Valley after the 110 mi<sup>2</sup> Owens Lake had been dried to support the water thirsty city of Los Angeles for a little more than a decade (1913-1926, feeding the Los Angeles aqueduct, see [60, 67]). Wilken et al. [78] indicated the inability of current dust mitigation strategies to protect construction workers from infections with Coccidioides spp. Lack of regulatory expertise and unrealistic expectations have resulted in costly failures of dust mitigation methods in the Western Mojave Desert in the past as described in McRae [52]. Environmental Impact Reports (EIRs) have been particularly criticized for not describing how dust mitigation measures are implemented and supervised, and no long-term dust control mitigation measures are included in the reports [73].

Mitigation and regulations are important considerations because some of the construction projects are in the immediate neighborhood of schools or close to human settlements. For example, the Del Sur Solar Project [Conditional Use Permit (Nos. 14-15 and 14-16)] is located adjacent to and upwind of Del Sur Elementary School. As of October 2012, the enrollment consisted of approximately 750 students in grades K-8 who would be directly and constantly affected by fugitive dust emissions because of daily westerly winds.

Although rarely implemented, potential mitigation procedures have been developed. Re-vegetation of disturbed land as a long-term strategy of dust control has been suggested by the Antelope Valley Dustbusters Taskforce, a group which consists of private entities, as well as federal, city, and county government representatives [3, 29, 41], but the implementation of their recommendations into Dust Control Plans (DCPs) rarely occurred [10]. Based on 20 years of dust mitigation experience in the Antelope Valley, The Dustbusters Task Force of 1991 developed handbooks for farmer and homeowners in the Antelope Valley which are publicly available at (http:// www.kernair.org/Main\_Pages/information.html#; see also [18, 29, 61, 68]). Based on the findings of this study, we recommend that EIRs include soil analyses for *Coccidioides* spp. on land destined for construction of any type in endemic areas of the pathogen.

## Conclusion

Although the change from non-renewable to renewable energy is generally welcomed in California, disturbing soils in endemic areas of a soil-borne pathogen that already causes disease incidence of epidemic character should only be considered if successful long-term dust mitigation measures are implemented, supervised, and controlled. The increasing demand for renewable energy shows promise for our planet in the future and will reduce some airborne emissions. However, there are hazards when sourcing new locations. One such danger is *Coccidioides* spp. arthroconidia becoming airborne when soil is disturbed and dust mitigation measures are inefficient or absent.

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#### **Compliance with Ethical Standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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