

Airborne Fungi Associated With Dust, their Source Identification and Contribution in the Southwest of Iran

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Research Article

Keywords: dust, fungi, Source tracker, Bioaerosol, HYSPLIT model

Posted Date: June 29th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-606923/v1>

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Abstract

Dust events impose negative socio-economic, health, and environmental impacts in vulnerable areas and reflect physiochemical and biological characteristics of their sources. The purpose of this study was to assess the impacts and contribution of two dust sources on concentration and diversity of airborne fungi in one of the most frequent dusty area in the world. Air masses arriving at study area were assessed using ground wind rose and HYSPLIT model. To explore the relationship between fungi in dust sources and downwind area, the sampling was carried out from airborne dust in Arvand Free Zone as targets areas and soil of dried part of Hor-alazim and Shadegan wetlands as sources areas. The samples were analyzed in the lab to extract DNA, PCR and sequencing. The Raw DNA data were processed using Qiime virtual box to pick OTUs and taxonomy assignments. The most common fungi at the Genus level were in the order of *Penicillium* > *Aspergillus* > *Alternaria* > *Fusarium* > *Paradendryphiella* > *Talaromyces*. The similarity between air and soil fungal genera was investigated using richness and diversity indices estimation, phylogenetic tree, PCA analysis. results show the ambient fungi community structures in Hor alazim, and Shadegan dust sources were more similar to those on dusty days than non-dusty days. To quantify the contributions of known dust sources to airborne fungi, Source tracker model was used. Results show that the main known airborne fungi sources were the Hor-alazim in dusty and non-dusty days. This study's results can help managers to identify and prioritize dust sources in terms of fungal species.

1. Introduction

Dust event is a consequence of the wind erosion in which large amount of small particles enters the atmosphere and influenced the downwind area. Dust events impose negative socio-economic, health, and environmental impacts in vulnerable areas involving the Middle East (Middleton, 2017). The negative health impacts of dust were extensively assessed in previous studies. Dust may cause various diseases such as respiratory, cardiovascular, cardiopulmonary, and mental diseases (Griffin et al., 2007, Sandstrom and Forsberg, 2008, Goudie, 2014, Sprigg, 2016, Soleimani et al., 2020, Perez et al., 2012, Ma et al., 2016, Middleton, 2017).

The major dust sources in Middle East arise from degraded lands, dried agriculture and wetlands of Iraq and Syria, Sahara Desert, and Saudi Arabia's deserts during last two decades.

The dust particles reflect the biological and physiochemical characteristic of their dust sources and pathways. The origin and pathway of bioaerosol associated with air masses arriving at study were assessed with back trajectory models such as HYSPLIT model (Innocente et al. 2017; Mu et al. 2020). Previous studies widely assessed the short and long-range transports of bioaerosol (Seifried et al., 2015, Smith et al., 2012, Nenes et al., 2013, Maki et al., 2013, Jeon et al., 2011, Polymenakou et al., 2008, Mazar et al., 2016, Meola et al., 2015, Lympelopoulou et al., 2016., Hervàs et al., 2009; Yamaguchi et al .,2012). Brief extend transport of bacteria for the most parts was considered due to similarities between airborne

bacteria and bacteria from known nearby sources. Back trajectory models such as HYSPLIT were utilized in evaluating the long transport of bacteria.

Strong correlation has been reported between dust and total fungi concentrations. Abundance and diversity of fungi and bacteria were assessed to investigate the similarity between origin and downwind area. The results of these researches confirm that fungi and bacteria as bioaerosol could transport from dust sources to the downwind area (Chao et al. 2012; Yarahmadi et al. 2020).

Several approaches have been used to fungi identification in airborne dust including morphological and molecular approaches. Morphological approaches use macro- and micro characteristics of fungal genera or species (solimani et al. 2013; Goudarzi et al. 2016).

Molecular approaches include DNA extraction, PCR application and sequencing. The sequences were compared with the DNA sequences deposited in GenBank using the Basic Local Alignment Search Tool (BLAST) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). (Kakikawa et al. 2008; Yarahmadi et al. 2020)

Also, QIIME virtual boxes have been applied to analyses raw DNA sequencing data based on billions of sequences from tens of thousands of samples. QIIME analyses are including OUT picking, taxonomic assignment, phylogenetic tree and diversity analyses (Bolyen et al. 2019, Mu et al. 2020)

Investigation of Similarity between fungi in source and target area has been utilized Richness and Diversity indices differences, phylogenetic tree, PCA analysis and heatmap. There is little study about the source apportionments of airborne fungi. In these researches, Source Tracker using Bayesian method, estimate proportion of all source samples (Mu et al. 2020).

Previous studies, in south west of Iran, focused on airborne fungi identification using culture base approach and the relationship between dust and airborne fungi concentrations. (solimani et al. 2013; Goudarzi et al. 2016). But how biological characteristics of dust source influenced airborne fungi in downwind area not well studied.

The innovation of this research is quantifying impacts of dust sources on fungal composition of downwind area in the south west of Iran as one of the dustiest area in the world. To this purpose, following steps were carried out: The accurate fungi identification in dust source and downwind area using molecular approach, similarity investigation between origin and target area based on fungal richness and diversity indices and the dust sources contribution estimation to airborne fungi.

This study focuses on determining the effects of known dust sources on airborne fungi communities. Samples were collected from surface soil of possible sources (dried parts of Hoor Al-azim and Shadegan wetlands), and airborne dust from Arvand free zone, addressing the following questions: (1) what the composition and type of airborne fungi are? (2) What are the differences among airborne fungi at dusty and normal days? (3) What are the relationships among fungi in possible dust sources and airborne fungi? (4) Can the contributions of dust sources quantify in airborne fungi? (5) Can dust sources prioritize to prevent damage of dust events?

2. Materials And Methods

2.1. Study area

During last years, with Intensification and continuation of drought, dried parts of Hor-alazim and Shadgan wetlands become the most dynamically active dust sources in the southwest of Iran. With passing air masses over these dust sources, huge amount of dust entered to the atmosphere and impose many damages to the downwind area such as Arvand free zone. In this area, more than 30 percent of days are dusty days and in some times, the concentration of PM10 has been recorded more than 10000 $\mu\text{g}/\text{m}^3$.

In this study, to assess relationship between dust sources and airborne fungi associated with dust, surface soil of dried part of Hor-alazim and Shadgan wetlands were selected as sources and Arvand free zone was selected as target area.

2.2. Material and methods

Figure (2) shows the flowchart of this research and below sections describe utilized method including air mass assessing, sampling, Fungal identification, PCR sequencing, Source tracker.

2.2.1. HYSPLIT Model and local wind rose

To assess the long transport of airborne fungi, HYSPLIT (Draxler et al., 2009) model was used. HYSPLIT is the back trajectory model that can determine air masses arriving at the sampling site. Each season's back trajectories were estimated every 2 hours at three-level heights (10,500 and 1000m) during the 2019 year. To determine main air masses arriving at the study area, estimated back trajectories were clustered.

According the data of Abadan synoptic station, ground wind rose was determined using wind speed, direction and frequency.

2.2.2. Air sampling

Five sampling sites were selected in Arvand free zone during spring. Air sampling was performed to detect fungi by a microbial air sampler (Quick Take30) at a distance from 1.5 to 2 m above the ground. Airborne particulate matter, fungi, humidity, temperature, and ultraviolet radiation were recorded under USEPA standards (EPA, 1998). Sampling days were clustered into dusty and non dusty days according PM10 standard level.

2.2.3. Dust source sampling

Dried parts of Hor-alazim and Shadegan wetlands were one of the foremost dynamic dust sources in the southwest of Iran. In recent years, their action was quickened due to the broad dry season and improper water administration within the area. 15 samples were collected from every dust source's surface soil haphazardly. Every sample was taken at a depth of 0–15 cm, and each soil test was almost 1 kg.

2.2.4. Fungal identification

Morphological and molecular data were used to fungal identification. Different colonies grew on the nutrient agar medium. Fungal concentrations were also calculated according to Colony (CFU/m³) and morphological characteristics. Phenol chloroform strategy was utilized for DNA extraction. In brief, a few fungal spores were transferred to plates containing Saburo dextrose agar medium, and a 2-3-day colony was utilized for DNA separation. 10 µl of suspension from organism's colony was added in 300 µl of lysis buffer along with 300 µl of the glass bead. Seriously vortexing was performed for 3 to 5 minutes to break the fungi cell. Then an equal volume (300 µl) of phenol chloroform solution was added to each tube and mixed and centrifuged at 5000 g for 5 minutes. The supernatant was then transferred to a new tube and an equal volume of chloroform solution was added to it and then centrifuged at 5000 g for 5 minutes. The supernatant was removed again and 0.1 ml of the initial volume of 3 M sodium acetate with pH = 5.2 was added to it with absolute isopropanol alcohol with a volume of 2.5 times the extracted supernatant. After storage for one hour at -20 ° C for 10 minutes centrifuged at 12000 g. The supernatant was discarded and about 500 µl of 70% alcohol was added to the precipitate. After 10 minutes of centrifugation at 12000 g, the liquid was gently removed and finally, one hour after the precipitate dried in the open air, 50 µl of deionized distilled water was added to the precipitate. And mixed thoroughly for 5 minutes. The extracted DNAs were stored in a freezer at -20 ° C. The ITS regions of rDNA gene were amplified using primers ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA - 3') and ITS4 (5'- TCCTCCGCTTATTGATATGC - 3'). PCR reactions contained 10 µL of premix (Ampliqon, Denmark), 3 µL of DNA template, 1 µM of each primer, and enough water to reach a final reaction volume of 20 µL. Negative controls (water rather than fungi DNA) were included to each PCR. The blend was at first denatured at 95°C for 5 min, taken after by 35 cycles of temperature changes, counting: 94°C for 30 s, 56°C for 45 s, 72°C for 45 s, and a terminal expansion step of 72°C for 5 min. PCR items were isolated through electrophoresis on 1.5% agarose gels. Finally, PCR products were sent to lab for sequencing. The sequences were compared with the those in GenBank using the BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and QIIME. A phylogenetic tree was constructed using MEGA5 and QIIME.

2.2.5. statistical analysis

Spearman tests were used to examine the correlation between fungal density with and dust concentration. Kruskal-Wallis test was used for comparing the fungal density between sampling day and points. Mann-Whitney U test for comparing the fungal density in non-dusty and dusty conditions.

2.2.6. Similarity assessment between fungi in dust sources and downwind area

Qiime virtual box was utilized to performing microbiome investigation from raw DNA sequencing information. Qiime analysis involves demultiplexing and quality sifting, OTU picking, ordered task, and phylogenetic reproduction, and differing qualities investigations and visualizations. The vegan (Dixon, 2003) packages of R environment were utilized in our research to fungal genera differences and

community structures. Differences (Simpson and Shannon) and abundance (Expert and chao1) were evaluated in R for dust source and air samples. To evaluate the similarity between surface-soil and air samples, phylogenetic tree, principal components analysis (PCA) were used.

2.2.7. Dust sources contributions in airborne fungi

Source tracker (Knights et al., 2011) was utilized to survey contribution of probable dust sources in airborne fungi. In Source tracker, a Bayesian method uses to estimate proportion of all source samples that contribute to a given airborne sample. The uncertainty of this estimation determined with estimation of unknown sources.

3. Results

3 – 1 Assessing air masses arriving at study area using HYSPLIT model and ground wind rose

Figure (3) shows the clustered back trajectories of air masses arriving at Arvand free zone during 2019. For each seasons, back trajectory each 2 hours were evaluated utilizing HYSPLIT. Air mass directions at three level heights (500, 1000 and 1500 m) were appeared during spring Fig. (3a1, 3a2, and 3a3), summer Fig. (3b1, 3b2, and 3b3), autumn Fig. (3c1, 3c2, 3c3) and winter Fig. (3d1, 3d2, d33). In spring, more than 70% of air masses started from Iraq and Syria. Almost 24% of air masses come from domestic dust sources at 10 m level height Fig. (3a1). In summer, at 10 m height, more than 90% of air masses started from Iraq and Syria and almost 8% of air masses were from neighborhood masses Fig. (3b1). At 500 and 1000 m level height, Jordan was included to Iraq and Syria. In autumn, neighborhood air masses were almost 40% and Iraq and Syria were the most sources of air masses. In winter, almost 25% of air masses come from south and south east. Almost 5% begun from north of Africa. Due to figure (3), the prevailing air masses' directions at three-level heights during four seasons were northwestern. The most elevated percent of nearby air masses were in autumn that caused domestic dust events.

According wind rose Fig. (3C), the prevailing ground wind direction comes from northwestern which is consistent with HYSPLIT model results.

HYSPLIT model Results and ground wind rose show that the air masses could pass over the dried part of Hor alazim and Shadegan wetlands and could transport fungal species associated with dust particles to the Arvand free zone.

3.2. Relationship between total cultivable fungi and dust (PM₁₀ µg/m³) concentration

According to standard PM₁₀ concentration (150 µg/m³), sampling days were clustered into two dusty and non-dusty groups. The Mann-Whitney U test results showed a significant difference between the density and diversity of fungi in dusty and non-dusty days ($p < 0.05$). Kruskal-Wallis test results shows there are insignificant differences among the density and diversity of fungi in sampling points ($p > 0.05$).

To explore the relationship between dust and total cultivable fungi, the Spearman test was used. This analysis showed a strong positive correlation between PM10 ($r = 0.77$, $p = 0.01$) and PM2.5 ($r = 0.71$, $p = 0.02$) with total cultivable fungi. Due to this analysis, it is concluded increasing the concentration of dust increases the concentration of total cultivable fungi.

3.3. Diversity of total cultivable fungi in air and soil samples

Raw DNA sequencing data were analyzed utilizing Qiime virtual box. These analyses include OTU picking, taxonomic assignment. Figure (4) indicates the fungi community structures in surface-soil of dust sources and airborne dust samples at class (4A), Order (4B), Family (4C), and genus (4D) level. The foremost common fungi at Genus level were in order of *Penicillium* > *Aspergillus* > *Alternaria* > *Fusarium* > *Paradendryphiella* > *Talaromyces*. Figure (5) show phylogenetic tree of samples and similar species in air and soil. Table (1) indicates the abundance and diversity (mean \pm standard deviation) of fungi communities in airborne dust and surface-soil samples. Fungi differences and richness varied between air and soil samples ($p < 0.05$). Fungi abundance (Chao1 and ACE) and diversity (Shannon and Simpson) were higher in surface-soil samples than in air samples ($p < 0.05$). Airborne fungi differences and abundance were higher at dusty days than non-dusty days ($p < 0.05$). In any case, there was a small distinction in fungi richness and differences between the two sources.

Table (1) richness (chao1 and ACE) and diversity (Simpson and Shannon) in air and soil samples

	non dusty days	dusty days	Hor_alazim	Shadegan
S.chao1	5.50	6.50	21.00	12.00
se.chao1	2.52	1.27	8.02	5.95
S.ACE	10.00	7.14	30.00	18.64
se.ACE	0.95	1.00	1.64	1.89
Simpson	0.72	0.78	0.91	0.79
Se. Simpson	0.06	0.07	0.08	0.8
Shannon	1.33	1.63	2.43	1.77
Se. Shannon	0.10	0.12	0.18	0.14

3.2. Relationship between air samples and dust sources in terms of fungi community structure

Figure (6) indicates the connections in fungi communities between air samples and samples collected from known dust sources (Shadegan and Hor-alazim tests) at the genus level. Due to the results, the structures of fungal community in Hor alazim and Shadegan dust sources were more comparable to those in dusty days than non-dusty days.

3.3. The contributions of dust sources in airborne fungi

Source tracker uses OUT table obtained from QIIME that represent fungal genera and their abundance in each samples. In Source tracker, a Bayesian method uses to estimate proportion of all source samples that contribute to a given airborne sample. Figure (7) indicates the impacts of the two dust sources on airborne fungi at dusty and non-dusty days. The most known source of airborne fungi was the Hor-alazim dust source in dusty and non-dusty days. In dusty days, the contributions of hor-alazim and Shadegan dust sources in airborne fungi were almost 15% and 9%, respectively. In non-dusty days, the contributions of hor-alazim and Shadegan dust sources were almost 7% and 4%, respectively. Besides, there were some unknown sources of airborne fungi (accounting for 78 to 87%); this ratio was higher in non-dusty days.

4. Discussion

Long transport of airborne bacteria extensively studies using HYSPLIT back trajectory models (Innocente et al., 2017; Mu et al., 2020). To better understand unknown sources of airborne fungi, HYSPLIT back trajectory model was used. Back trajectories of each season were estimated every 2 hours during 2019. To assess main air masses directions, estimated back trajectories were clustered in each season in 10, 500, and 1000 m level heights. Results show that the dominant air masses directions were from North West, and they originated from Iraq, Syria, Jordan, and Saudi Arabia. These air masses may transport fungi by passing over foreign dust sources. Except for summer, the second main directions were from the southeast and may be associated to dust from domestic dust sources. The highest percent of local air masses were in autumn which may cause severe domestic dust storms. HYSPLIT model Results and local wind rose show that the air masses could pass over the dried part of Hor alazim and Shadegan wetlands and could transport fungal species associated with dust particales to the study area.

There was a significant difference between total cultivable fungi, PM10, and PM2.5 concentrations during dusty and non-dusty days. The average concentration of fungi in dust days (99.3 CFU/m³) was 1.7 times higher than those on typical days (58.4 CFU/m³). (Yarahmadi et al., 2020) detailed that the average concentration of fungi in dust days (967.65 CFU/m³) was 3.6 times higher than those in on ordinary days (267.10 CFU/m³) in Khorammabad territory, Iran. Furthermore, there was a strong correlation between dust concentrations (PM10 and PM2.5) and fungi. This correlation was little more among the concentrations of fungi and PM10 than the concentrations of fungi and PM2.5. Thus, it is concluded that increasing PM10 levels led to an increase in fungi concentrations. Pervious studies founded a strong relation between PM10 with bacterial and fungal organisms in normal and dusty conditions (p-value < 0.001) (Nourmoradi et al., 2015; Goudarzi et al. 2014; Solimani et al. 2016).

The most common fungi at the Genus level in the present study were in the order of *Penicillium* > *Aspergillus* > *Alternaria* > *Fusarium* > *Paradendryphiella* > *Talaromyces*.

Bacillus genera among bacteria and Aspergillus spp. among fungi were reported to be the most common microorganisms during periods of dust storms in Iraq (Al-Dabbas et al., 2012), Saudi Arabian (Kwaasi,

2003), and Iran (Najafi et al., 2014) Solimani et al. 2013 reported that the dominant fungal genera were *Aspergillus sp.* *Alternaria sp.* *Cladosporium sp.* *Penicillium sp.* *Rhizopus sp.* in Ahvaz. (Mazloomi et al., 2017) reported that dominant fungal genera were *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria* in Ilam.

Table (1) shows the richness and diversity (mean \pm standard deviation) of fungi communities in ambient PM10 and surface-soil samples in probable dust source regions. Environmental stress such as high temperature, UV radiation, and low humidity may cause lower richness and diversity of airborne fungal genera than those from dust sources. Furthermore, the richness and diversity of dusty days were higher than non-dusty days because there is a strong relationship between total fungi and dust concentrations, and dust events may be associated to other fungal genera and may change the mycobiota in downwind. Yarahmadi et al., 2019 reported that 61 and 45 species were detected in Khoramabad during normal and dust days, respectively.

PCA analysis was used to explore the relationship between airborne fungi and those in dust sources. Results show the ambient fungi community structures in Hor alazim, and Shadegan dust sources were more similar to those in dusty days than non-dusty days. Thus, it is concluded that some proportions of total fungi are related to dried parts of Hor-alazim and Shadegan wetlands, and airborne fungi could reflect fungal genera from known dust sources. Yamaguchi et al. (2012) Asian dust could reflect the microorganism type and composition of their dust sources. MU et al. (2020) showed the similarity between airborne bacterial community samples and those from their possible local sources.

To quantify the contributions of known dust sources in airborne fungi, Source Tracker was used. Results show that the main known sources of airborne fungi were the Hor-alazim dust source in dusty and non-dusty days. In dusty days, the contributions of hor-alazim and Shadegan dust sources were about 15% and 9%, respectively. On non-dusty days, these contributions were lower. Besides, there were some unknown sources of airborne fungi (accounting from 78 to 87%); this proportion was higher on non-dusty days. To reduce the unknown proportion, other sources should be identified.

5. Conclusion

HYSPLIT model Results show that the air masses could pass over the dried part of Hor alazim and Shadegan wetland and fungal species as bioaerosol could transport to the study area. Results of richness, diversity and PCA analyses confirm the similarity between soil and airborne fungal species. The dominant fungal genera were *Penicillium*, *Aspergillus*, *Alternaria*, *Fusarium*. These species can survive in adverse environmental conditions and have been reported as frequent species in Iraq, Syria, Jordan, and Saudi Arabia.

Dust source control is the most effective way to prevent humans and ecosystems from dust damages and dust sources identification is the first step of wind erosion control. Furthermore, determining the contributions of sources to airborne particles is needed to prioritize dust sources. Source Tracker results show that Hor-alazim dust source have more contribution to airborne fungi than Shadegan dust source.

In dusty days, the amounts of dust sources contribution will be increased. This study's results can help managers identify and prioritize dust sources in terms of fungal species.

Declarations

Acknowledgement

This research study is a part of MSc. thesis of Forough Tajiki which is financially supported by Khorramshahr University of Marine Sciences and Technology, Khorramshahr, Iran.

Ethical Approval: Not applicable

Consent to Participate: Not applicable

Consent to Publish: Not applicable

Authors Contributions: Forough Tajikei conducted the experiments and data curation. Hossein Mohammad Asgari and Isaac Zamani analyzed the results. Forough Tajikei and Hossein Mohammad Asgari, Farshid Ghanbari reviewed and edited the manuscript, Hossein Mohammad Asgari and Farshid Ghanbari designed the study. All authors read and approved the final manuscript.

Funding: This study was financially supported by the grant of the office of Vice chancellor for Research at Khorramshahr university of Marine Science and Technology, Khorramshahr, Iran.

Availability of data and Materials: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Figures

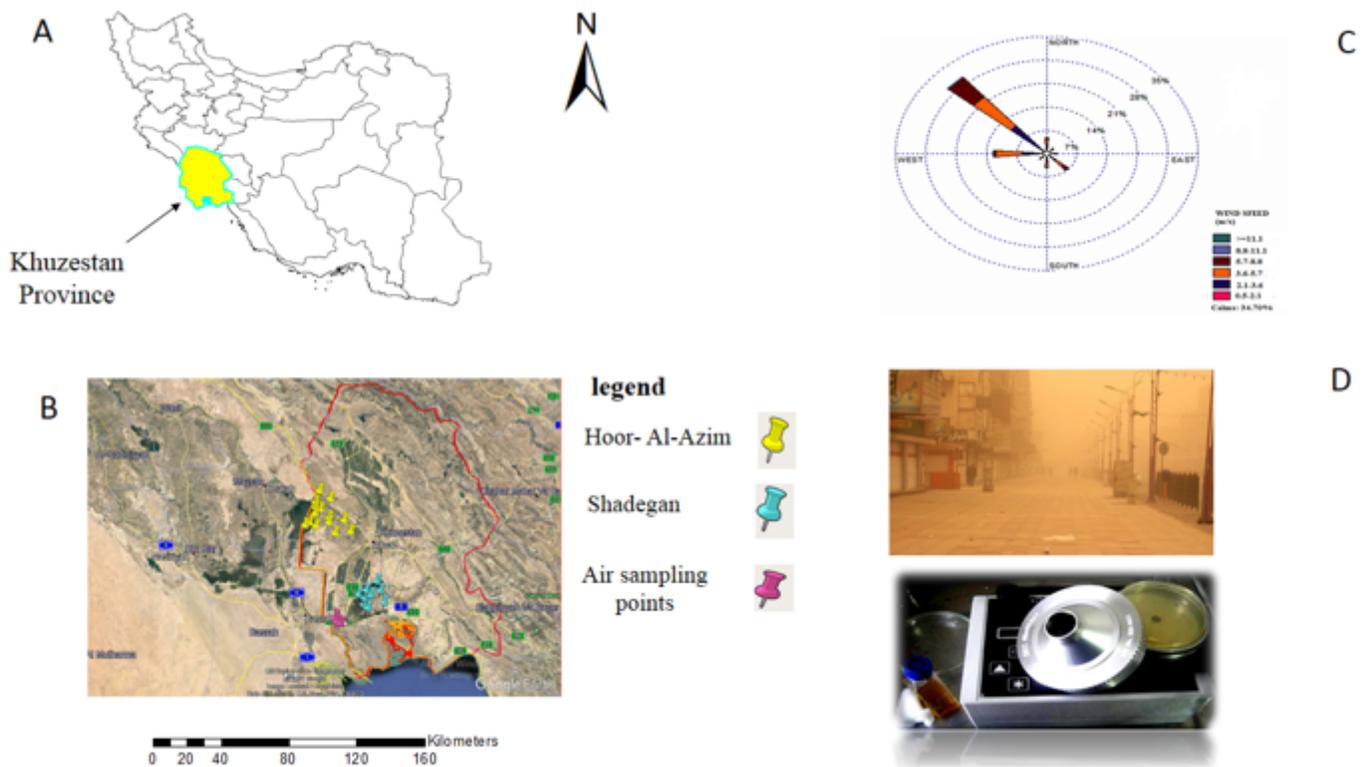


Figure 1

study area (A), and sampling points (B), local wind rose (C), dusty days in Khuzestan province (D)

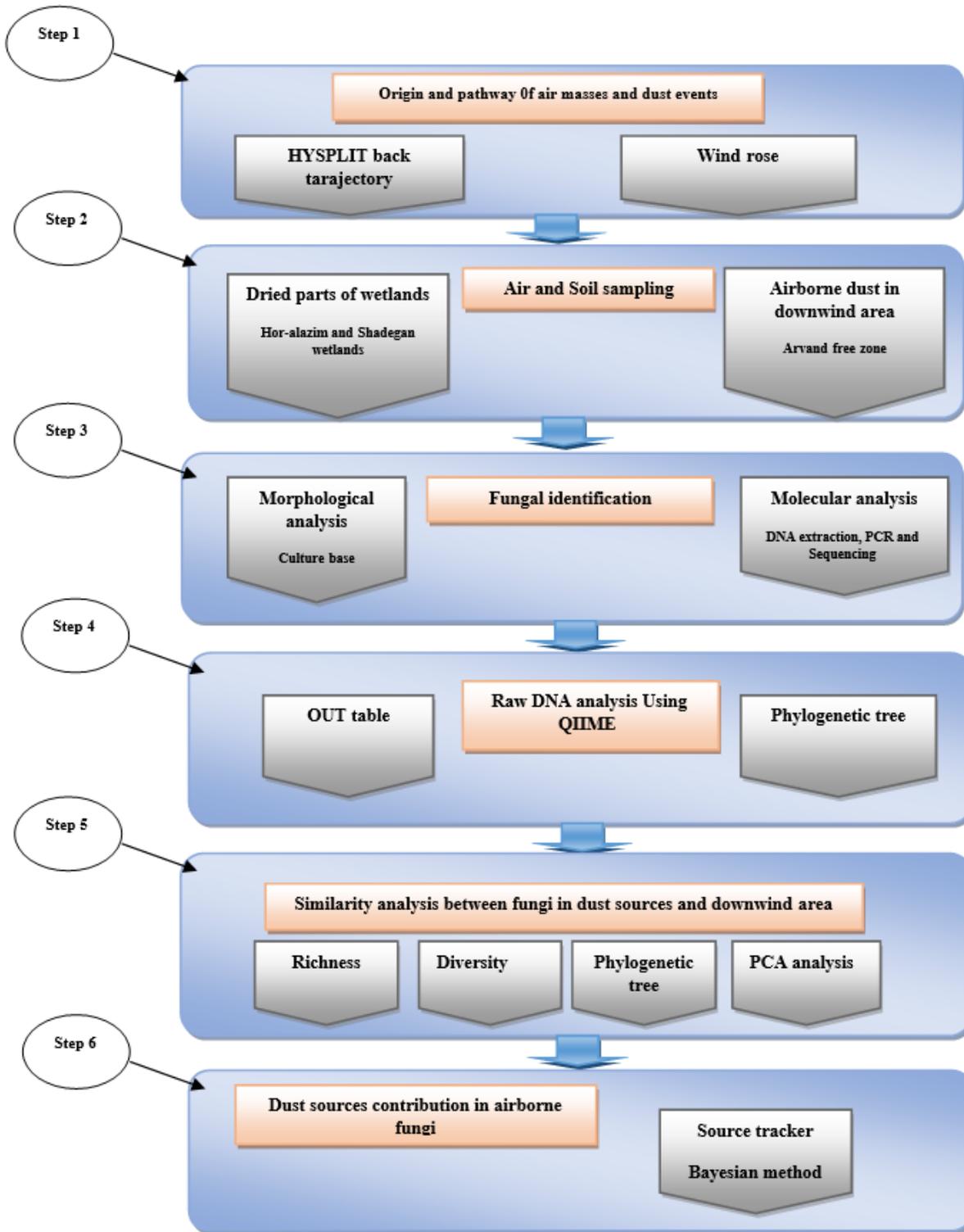


Figure 2

The methodology flowchart

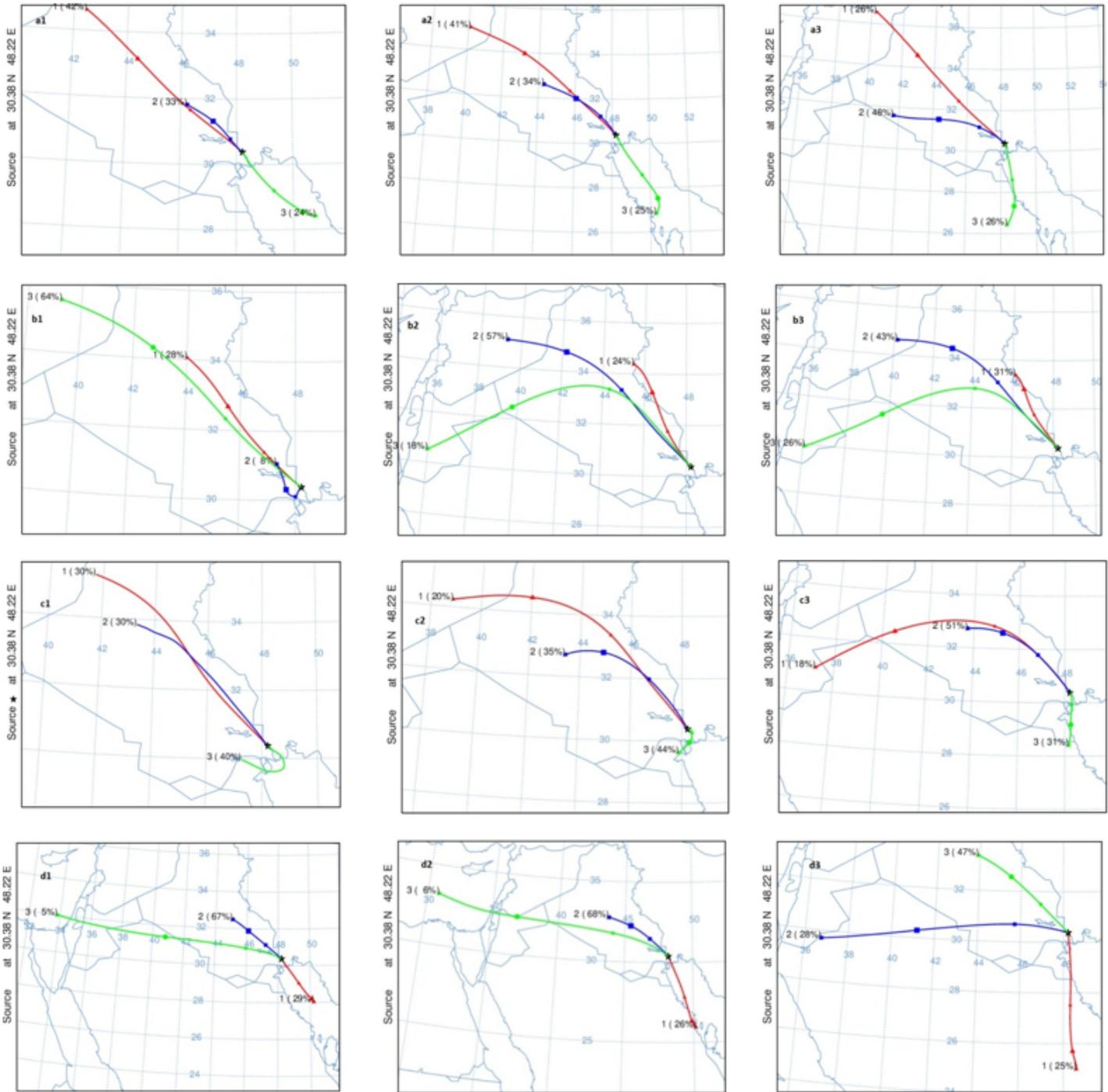


Figure 3

clustered back trajectories of the air masses arriving at Abadan site during 2019: spring (a), summer (b), autumn (c) and winter (d) at 10 m (a1, b1, c1 and d1), 500 m (a2, b2, c2 and d2) and 1000 m (a3, b3, c3 and d3) level heights

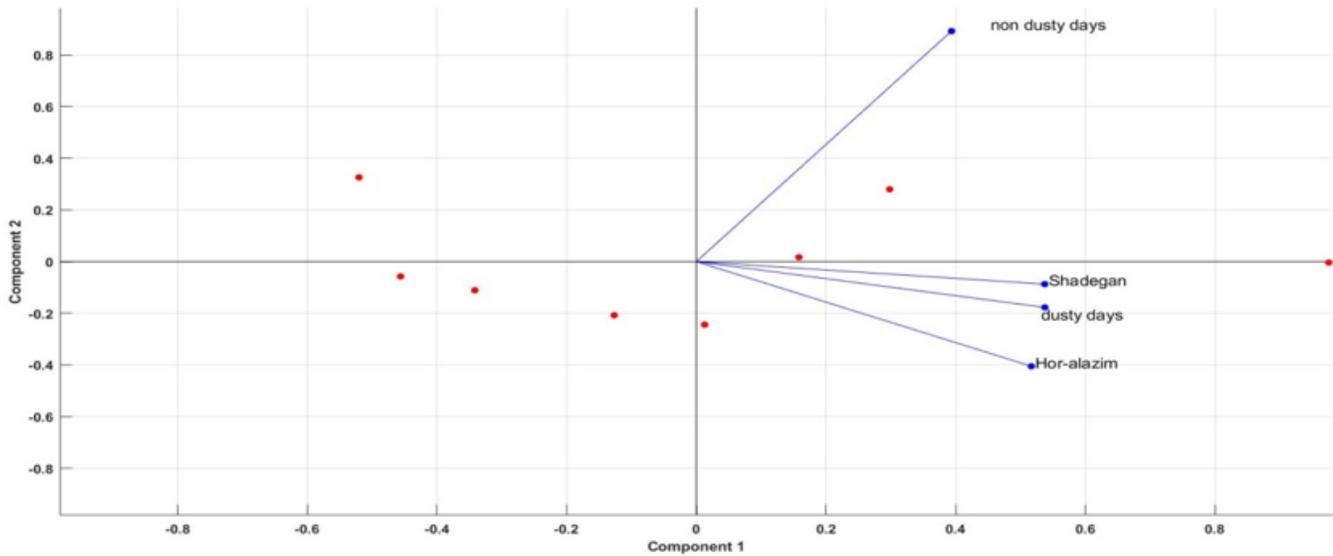


Figure 6

Relationships in fungal communities between air samples and samples collected from possible dust sources (Shadegan and Hor-alazim samples) at the genus level

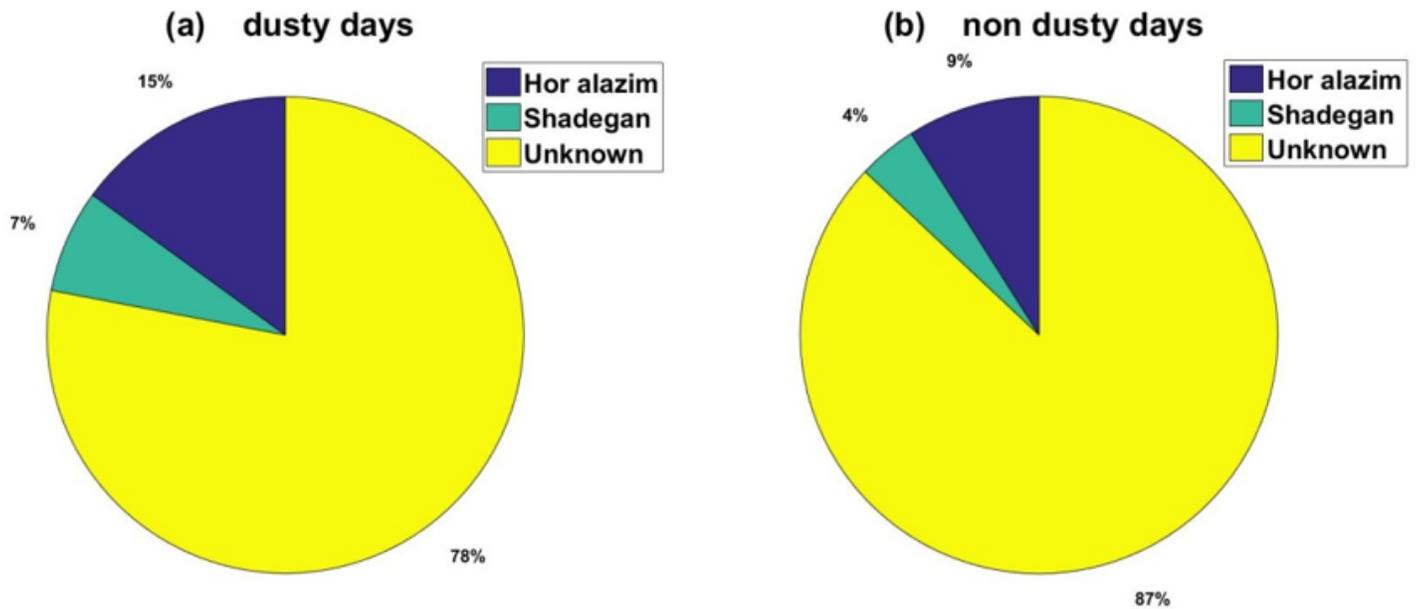


Figure 7

Estimated contributions of dust sources on airborne fungi in dusty and non dusty days