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Identification of Fungi and Yeasts from the Sands of the Pyramids of Giza, in Cairo, Egypt

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Abstract:
The pyramids in Egypt have many visitors annually, especially during the summer. Many tourists get sick, probably from being exposed to the wind, which mostly contains sand particles. The samples were collected in July 2019. The objective of this research was to know the diversity and quantity of filamentous fungi and yeasts in the dry sands of the pyramids. To determine if the fungi found are pathogenic for man. One gram of sample was taken in triplicate and spread on each plate with culture medium; RBA, SDA, PDA, and Hardy ChRom. The samples were incubated for 7 to 14 days at 25 ° C. Colonies were counted and then species were isolated in tubes of each medium. Six genera were found: *Aspergillus, Penicillium, Rhizopus, Candida, Alternaria,* and *Hortaea*. The *Aspergillus* and *Candida* species showed more growth. The average of filamentous fungi and yeasts in the samples ranged from 13 CFU / g to 21 CFU / g. This indicates that the sand according to the results would be of average quality. The species identified were *A. niger, A. flavus, R. stolonifer, P. raistrickii, Hortaea werneckii, Alternaria, C. glabrata,* and *C. tropicalis*. Most of the yeasts and filamentous fungi identified are pathogenic to humans. Some of the identified species develop several diseases, so people with weak immune systems will need to protect themselves. It is advisable to cover the mouth, nose, and wear glasses for the eyes so that the spores cannot access the chemical and physical barriers of our body.

INTRODUCTION

The pyramids at Giza suffer from a lot of Geo-environmental and structural problems including topography, geology, climate, and human actions that seem to have a significant impact on environmental processes (Hemeda and Sonbol, 2020). The pyramids at Giza are subjected to terrestrial and human influence and are vulnerable to microbial deterioration; since the prevailing environmental conditions of temperature and humidity are more suitable for microbial growth in tropical and subtropical regions of the world (Evers, 1929). Sand is a complex environment with a diverse range of microorganisms to proliferate despite the harsh conditions due to low water availability and nutrient concentration (Echevarría, 2019a; Iqbal *et al.*, 2019; Rizk *et al.*, 2016).

Many fungal species are potentially pathogenic, as opportunistic pathogens, infecting primarily the immunocompromised, or virulent parasites of individuals with normal immune systems (Echevarría, 2019b). Several studies have documented that dry sand host a diverse range of organisms, such as small invertebrates, bacteria, fungi, virus, algae, and diatoms that can adapt constantly changing environments (Echevarría, 2019b; Shah *et al.*, 2011; Velonakis *et al.*, 2014; Whitman *et al.*, 2014). The sandstorms facilitate the long-distance dispersal of dust-associated biological particles includes bacteria, fungi, and viruses, and these organisms can be carried across several continents (Goudie and Middleton, 2001).

However, the microbial communities of Egyptian pyramid zones are poorly investigated (Abdel-Azeem and Salem Fatma, 2013). These zones represent a dynamic environment, characterized by large sand deposits, rapid morphological changes due to a large number of visitors. The medical risk to human health arises from the fungi associated with sandstorm dust-producing metabolites (mycotoxins) that can initiate a toxic response to humans (Griffin, 2007). Several fungi and yeast species may behave as opportunistic or pathogenic becoming a potential risk for human health by producing several mycotoxins (Al-Jobory *et al.*, 2017; Humaid *et al.*, 2019; Iqbal *et al.*,, 2019; Iqbal *et al.*, 2018; Krüger *et al.*, 2019). Nevertheless, microorganism communities at the pyramids have been often neglected and real health risks have not been assessed.

The impact of severe sandstorm dust events on local air quality and public health has become of greater concern in the Egyptian pyramid zones in recent years. Recently, the study of such communities has received greater attention in view of the safety of pyramid zone environments since they are intensely visited areas. Therefore, the investigation of the dry sand fungi at pyramid zones results are essential to know the related mycological biodiversity, to prevent potential sources of pollution, and to assess the risks of potential contaminations by opportunistic, pathogenic, or allergenic fungi and health risk to the visitors.

The main objective of this study is to characterize the Fungal and Yeasts diversity from the Sands of the Pyramids of Giza, in Cairo, Egypt.

MATERIALS AND METHODS

Sampling and culture media

The sands were obtained from the dry zone of the pyramids (Figure 1), collected in three equidistant points, and stored in sterile plastic bags. The agar used to culture the sand was Rose Bengal Agar (RBA), Potatoes Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA) and Hardy ChROM agar was used for further isolation of the colonies in tubes. To demonstrate that the medium can grow, the agar was inoculated on a plate with *Aspergillus fumigatum* (positive control) and another plate without organism, to guarantee the sterility of the medium (negative control), these inoculated in both media (RBA, SDA, PDA, and Hardy ChROM agar). The growth of the pure culture was performed at 25 °C for 7 to 14 days in the incubator. Pure culture and isolation of colonies: 1gram of the sand of each beach was weighed in triplicate, spread on a Petri dish with half RBA, SDA, PDA, and Hardy ChROM agar (Echevarría, 2017). Incubation was then performed at 25 ° C for 7 to 14 days. After this

period isolation of colonies of fungal filaments was made using PDA tubes (Jorge Luna, 2012), RBA, SDA, and Hardy ChROM agar.

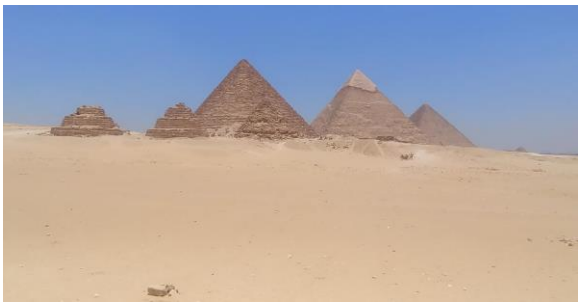

Fig. 1. Sampling location at pyramids in Egypt.

Counting and average of colony-forming units (CFU)

To determine the quality of the sands of pyramides Giza Cairo Egipt, it was estimated through (CFU) colony forming units (Forbes, 2009). According to the total number of colonies, the quality of the sands was determined using the maximum values recommended by the National Institute of Saúde Ricardo Jorge (INSA) (Brandão *et al.*, 2011) and the National Health Institute on Portugal (Pereira *et al.*, 2013). The values recommended by the institute table 1 were used to determine the quality of Cairo Egypt sands. The beaches studied in Portugal have characteristics and an environment like the pyramids Giza sands (Pereira *et al.,*, 2013).

Macroscopic and microscopic identification

The identification of genus and species was achieved after a morphological study both macroscopic and microscopic. For the macroscopic morphology, the color and appearance of the surface and the back of each sample were observed. To study the microscopic morphology, samples from the isolated colonies were transferred to a slide with Lactophenol. They were observed in the Nikon Eclipse Ci microscope. The data obtained were compared, using taxonomic keys.

RESULTS AND DISCUSSION

Counting colony-forming units and sands quality

The approximate average of the number of total colonies was calculated to determine the quality of the sands of the pyramids of Cairo and compared with the standards following the criteria of the INSA (Table 1). The average of the three samples was obtained. According to the results, 21 CFU / g was obtained in the samples with the SDA medium. On the other hand, with the PDA medium, 13 CFU / g was obtained and with the RBA medium 16 CFU / g was obtained. The average colony-forming unit count was 17 CFU / g for pyramid sand in Egypt. We also use HardyCHROM medium, with which we measure yeast growth. The average of the yeasts was 18 CFU / g. The sands of the pyramids in Cairo, Egypt, showed an (MRV) average quality (Table 2). A previous study reported the counts of filamentous fungi in the sand samples ranged from 6 CFU / g to 17 CFU / g (Echevarría, 2019b).

Table 1. Values of fungal count recommended by the National Institute of Saúde Ricardo Jorge and the National Health Institute on Portugal (Pereira *et al.,*, 2013).

Standard Values to Determine the Quality of Sands		
> MVA poor quality	> MRV average quality	≤ MAV good quality
CFU/g = 85	CFU/g = 5	CFU/g = 5

Table 2. Mean counts of the fungal species from the sand samples from dry zones of pyramids.

Sr. No.	Fungal species	Media used	Mean fungal count (CFU/g)
1.		SDA medium	21
2.	Fungi	PDA medium	13
3.		RBA medium	16
4.	Yeast	HardyCHROM medium	18

The count allows us to determine the quality of the sand, using this study as a specification and from the result to know if the sands of the pyramids, a tourist area in Cairo, could be a concern for public health.

Species of filamentous fungi

Two species of fungi of the genus Aspergillus were identified. In total, the filamentous fungi species found in the sands were *Rhizopus stolonifer, Aspergillus niger, Penicillium raistrickii, yeast sp, Candida tropicalis, Candida glabrata, Alternaria sp., Aspergillus flavus,* and *Hortea wernekii* (Table 3).

This study verified that the fungal species found are mostly pathogenic for humans, such as Aspergillus and Penicillium species among others, which are a possible risk to public health.

In Puerto Rico, an investigation in the sands of the beaches of the northern zone of Puerto Rico identified six genera of filamentous fungi: Aspergillus, Penicillium, Rhizopus, Trichoderma, Hortaea, and Fusarium (Echevarría, 2017). In another study, a variety of pathogens were reported from beach sands, and some evidence of health risks associated with sand exposure (Whitman *et al.,*, 2014).

Table 3. Isolated fungi and diseases that cause.

Fungal species	Disease	Reference
Rhizopus stolonifer	Allergic alveolitis	(Pontón *et al.,* 2002)
Aspergillus flavus	Pulmonary infections	(Samson *et al.,* 2014)
Penicillium rastrickii	Hongo endófito, asociado con el suelo y en la descomposición de materia orgánica.	(Pitt and Hocking, 2009)
Aspergillus niger	Frequent agent of aspergilloma. Causes skin conditions and respiratory and disseminated infections particularly in immunocompromised patients. Aspergilloma.	(St-Germain and Summerbell, 2011)
Candida glabrata	The second most frequently isolated Candida species after C. albicans. Often involved in urogenital infection.	
Candida tropicales	A cause of bloodstream infection is wound infection following major surgery and disseminated infection. Mostly seen in patients with impaired immunity.	
Hortea wernekii	Ringworm disease, superficial fungal infection of the skin. It usually occurs in the palm and the soles of the feet. The lesions are not inflammatory.	(Kejžar *et al.,* 2013)
Alternaria	It mainly infects immunosuppressed hosts. Although infections in immunocompetent hosts have also been reported. The portal of entry for infection is usually through corneal trauma or breakdown of the skin barrier. Most of the clinical manifestations involve the skin and subcutaneous infections, although other types of infections. Eg Oculomycosis, sinusitis, onychomycosis, and invasive disease.	(Gilaberte *et al.,* 2005) (Pastor and Guarro, 2008)

In this case, the sand studied is of poor quality. According to other research, this study provides vital information to be able to carry out a more comprehensive study of the sands of Cairo in Egypt, in a whole year. This is to be able to determine if the same species of fungi are found throughout the year or there is a time when it is much less. Also being able to see if solid waste pollution or weather increases or decreases fungal growth. Table 3 shows the diseases caused by some of the fungi found in the study.

CONCLUSION

Based on the results, it can be determined that the sand from the pyramids would be classified as average quality. The growth of filamentous fungal colonies in the samples was from 13 CFU / g to 21 CFU / g. In the taxonomy analysis, six genera were found: *Aspergillus, Penicillium, Rhizopus, Alternaria, Hortea,* and *Candida.* The fungus genera with the most identified species were Aspergillus and Candida. The identified species were nine; *A. niger, A. flavus, R. stolonifer, P. raistrickii, Hortea werneckii, Alternaria, C. glabrata,* and *C. tropicali*.

Most of the yeasts and filamentous fungi identified are pathogenic to humans. Some of the identified species develop several diseases, so people with weak immune systems will need to protect themselves. It is advisable to cover the mouth, nose, and wear glasses for the eyes so that the spores cannot access the chemical and physical barriers of our body.

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CONFLICT OF INTEREST

The authors declare that this article's content has no conflict of interest.

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