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Fungal Contamination of Sandpits from Recreational Parks and Schools: A Potential Risk for Human Health

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Abstract

Sandpits used by children are frequently visited by wild life which constitutes a source of fungal pathogens and allergenic fungi. This study aimed to take an unannounced snapshot of the urban levels of fungal contaminants in sands, using for this purpose two public recreational parks, three elementary schools and two kindergartens. All samples were from Lisbon and neighboring municipalities and were tested for fungi of clinical interest.

Potentially pathogenic fungi were isolated from all samples besides one. *Fusarium dimerum* (32.4%) was found to be the dominant species in one park and *Chrysonilia* spp. in the other (46.6%). Fourteen different species and genera were detected and no dermatophytes were found. Of a total of 14 species and genera, the fungi most isolated from the samples of the elementary schools were *Penicillium* spp. (74%), *Cladophialophora* spp. (38%) and *Cladosporium* spp. (90%). Five dominant species and genera were isolated from the kindergartens. *Penicillium* spp. was the only genus isolated in one, though with remarkably high counts (32500 colony forming units per gram). In the other kindergarten *Penicillium* spp. were also the most abundant species, occupying 69% of all the fungi found.

All of the samples exceeded the Maximum Recommended Value (MRV) for beach sand defined by Brandão et al. 2011, which are currently the only quantitative guidelines available for the same matrix. The fungi found confirm the potential risk of exposure of children to keratinophilic fungi and demonstrates that regular cleaning or replacing of sand needs to be implemented in order to minimize contamination.

Keywords: Recreational Parks; Elementary Schools; Kindergartens; Sand; Fungal Contamination

Intoduction

Soils rich in keratin residue and other organic debris constitute a permanent or occasional reservoir for dermatophytes and other potentially pathogenic fungi. Superficial mycoses such as dermatomycoses are the most common human fungal infections produced by dermatophyte fungi that belong to three genera: *Trichophyton, Microsporum* and *Epidermophyton* which affect the skin, hair and nails [1]. The frequency of fungal infections are especially high in urban environments where people, waste, wild and domestic life, congregate in larger densities [2].

Sandpits used by children are frequently visited by animals such as sparrows, pigeons, dogs, cats and rats and constitute as a source for fungi [2]. Despite this knowledge, standards for fungal quality assessment of sandpit sand still lack regulations by international environmental and health agencies or even local and regional agencies. Municipalities in Portugal are, nevertheless, legally obligated to maintain recreational parks at good hygienic and safety levels, which include annual sand replacement, when used.

A number of studies detected a diverse number of species of fungi in beach sand, an equivalent matrix despite the differences in recreational contexts [3-6]. Under natural conditions, UV light affects both bacteria and fungi but fungi appear to be less susceptible and remain viable for longer periods of time [7]. Some fungi remained thus viable in sand under UV light exposure in lab conditions for up to 6 months [7].

In absence of guidelines and levels of fungal contaminants, the assessing system proposed by Sabino et al., 2011 [3] and by Brandão et al., 2011 [8] was considered as compatible, given the matrix similarities. In this system, three different fungal groups are searched: (1) Dermatophytes, (2) Potentially pathogenic and allergogenic fungi and (3) Yeasts, as explained by the authors. Group 2, the most heterogeneous one include aerial sporulating species such as the *Aspergillus fumigatus* and *Aspergillus niger* complexes and the genera *Aspergillus* spp. (unspecified species). Also *Fusarium* spp., *Neoscytallidium* spp., *Scopulariopsis* spp. and *Scedosporium* spp. and any other species or genera which counts results are above 500 CFU per gram of sand are included [3, 8].

A pilot study of sandpits was designed in children's playgrounds to assess fungal contamination levels in sand from two public recreational parks, three elementary schools and two kindergartens in the region of Lisbon and Tagus Valey.

Materials and Methods

The study was designed in children's playgrounds and includes two public recreational parks, three elementary schools and two kindergartens of the Lisbon and Surrounding Municipalities. The playgrounds were chosen due to their poor hygienic conditions trying to characterize the most critical scenario regarding exposure of children attending.

A composite of three *loci* samples from each location of up to 10 centimeter deep was collected with sterile gloves into a sterile plastic container and transported to the laboratory for analysis within 5 days (to ensure viability of all fungi present). For the culture method, 40 g of each sample (composite) were suspended in 40 ml sterilized water, followed by agitation during 30 min at 100 rpm. Duplicates of 0.2 ml for each sample were inoculated in malt extract agar (2%) with chloramphenicol (0.05 g/L) and incubated during 5 to 7 days at 27.5 ºC and 40 ºC.

For quantification purposes, and regardless of the microbe recovery (extraction) levels, 1 ml sand wash was presumed to represent 1g sand, given the 1:1 extraction ratio. An additional incubation at 40ºC was used to specifically select the growth of *Aspergillus fumigatus sensu stricto*.

Vanbreuseghem's (1952) [9] hair-bait technique was used in order to detect dermatophytes .After exposure of sand during 20 days at 27.5ºC baby sterilized hair were transposed to agar mycosel plates with cyclohexamide, at the same temperature for two weeks.

For species identification, microscopic mounts were performed using tease mount or Scotch tape mount and lactophenol cotton blue mount procedures. Morphological identification was achieved through macro- and microscopic characteristics according to de Hoog et al. (2001) [10]. Fungi were identified at the species level whenever possible, since adverse health effects tend to vary within genera [10,11].

Statistical Analysis

The data analysis was performed and used descriptive statistics using frequency, median and graphical representations appropriate to the nature of the data.

Results

The quantification results for fungi obtained from each analyzed sand sample are summarized in Table 1 (recreational parks) and Table 2 (schools).

Recreational parks

Fourteen different species/genera were obtained from both recreational parks. In Sample 1 (Recreational Park 1), 10 fungal species were identified in plates incubated at 27.5ºC, being *Fusarium dimerum* (complex) (32.4%), *Cladosporium* spp. (23.5%), *Fusarium solani* (complex) and *Phoma* spp. (11.8%) the most frequently isolated (Table 1). In addition to these species, the following were also identified: *Chrysosporium* spp., *Penicillium* spp., *Alternaria* spp., *Geomyces* spp., *Fusarium* spp. and *Aspergillus sydowii.* The same samples incubated at 40ºC

exhibited only *Alternaria* spp.

Table 1. Fungal distribution of the samples collected from the two recreational parks (incubation at 27.5^{0}C).

Sample 1			Sample 2		
Fungi	Freq. (%)	CFU/g	**Fungi**	Freq. (%)	CFU/g
F. dimerum (complex)	32.4	55	*Chrysonilia* sp.	46.6	170
Cladosporium sp.	23.5	40	*Cladosporium* sp.	28.8	105
F. solani (complex)	11.8	20	*Acremonium* sp.	15.1	55
Phoma sp.	11.8	20	*Fusarium* sp.	4.1	15
Other species or genera	20.5	35	Other species or genera	5.4	20
Total		170	Total		365

From Sample 2 (Recreational Park 2), when incubated at 27.5ºC, 8 fungal species were isolated, *Chrysonilia* spp. (46.6%) the most prevalent, followed by *Cladosporium* spp. (28.8%) and *Acremonium* spp. (15.1%) (Table 1). The same sample, incubated at 40ºC, showed growth of *Penicillium* spp. as the most prevalent genera (59.3%), followed by *Scopulariopsis* spp. and *Phialophora* spp. (14.8%). No dermatophytes were detected.

Schools

Fourteen fungal species were isolated from samples collected from elementary schools (Samples 1, 2 and 3 - incubated at 27.5ºC - Table 2). In Sample 1, *Penicillium* spp. (74%) was the most frequently found, followed by *Fusarium* spp. (22%) and *A. niger* complex (4%). In Sample 2 *Cladophialophora* spp. (38%), *Fusarium* spp. (23%) and *Neoscytalidium* spp. (23%) were the most frequent. In addition to these species, *Aspergillus ustus* complex and *Exophiala* spp. were also identified. In Sample 3, *Cladosporium* spp. was the most prevalent (90%). Other species were also found, namely: *Fusarium* spp., *Cladophialophora* spp., *Phoma* spp., *Alternaria* spp., *Rhizopus* spp., as well as isolates from *A. terreus* complex *and A. flavus* complex.

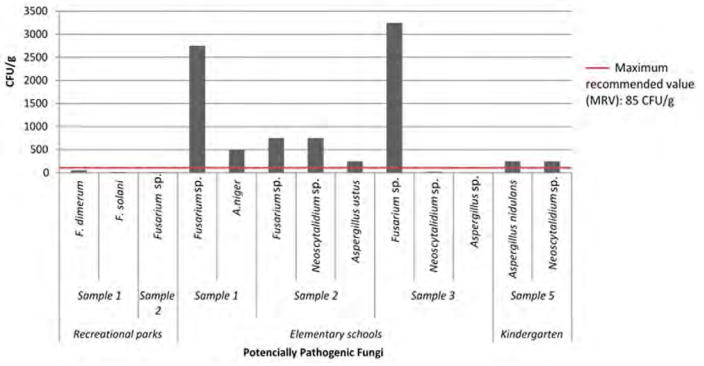
When samples from kindergartens (Samples 4 and 5) were incubated at 27.5ºC, 5 different fungal species/genera were isolated (Table 2). *Penicillium* spp. was the only genera isolated from Sample 4 and in higher counts than the other samples (Table 2). In Sample 5, *Penicillium* spp. was also the most frequently identified (69%), followed by *Phoma* spp. (13%), *Acremonium* spp., and *Neoscytalidium* spp. and *Aspergillus nidulans* (complex) (6%). Sample 4 was the only one that did not present any fungal species that belongs to the potentially pathogenic fungi group. All samples showed a CFU counting above the Maximum Recommended Value (MRV) described for beach sand in Brandão et al. (2011) [8] (Figure 1).

Table 2. Fungal distribution in of the analyzed samples collected from schools' sandpits (incubation at 27.5 ^{0}C).

Samples		Fungi	Freq. (%)	CFU/g
Elementary schools	1	*Penicillium* sp.	74.0	9250
		Fusarium sp.	22.0	2750
		A.niger (complex)	4.0	500
	2	*Cladophialophora* sp.	38.0	500
		Fusarium sp.	23.0	750
		Neoscytalidium sp.	23.0	750
		Aspergillus ustus (complex)	8.0	250
		Others	8.0	250
	3	*Cladosporium* sp.	90.0	46000
		Fusarium sp.	6.0	3250
		*Cladophialophor*a sp.	2.0	775
		Others	2.0	540

Kindergarten	4	*Penicillium* sp.	100	32500
	5	*Penicillium* sp.	69.0	2750
		Phoma sp.	13.0	500
		Aspergillus nidulans (complex)	6.0	250
		*Neoscytalidiu*m sp.	6.0	250
		Acremonium sp.	6.0	250

Figure 1. Potential pathogenic fungi distribution from the analysed sand samples.



Discussion

In urban areas, where there are high concentrations of people and animals, soil rich in organic matter may constitute a permanent or occasional reservoir for fungi. These fungi can be a potential source of skin infections for humans and animals [2]. Therefore, isolation of fungi from public parks sandpits may be a cause for concern. The risk of fungal skin infection is greater for children, a susceptible age group playing in sandy playgrounds [12]. Sandpits are thought to play a role in the epidemiology of human and animal mycoses [13].

Brandão and colleagues (2011) [8] proposed a value of 85 CFU/g as MRV for potentially pathogenic fungi, based on averages of an extensive national representation. In the present study a fungal load from all the identified genera exceeds the MRV for potential pathogenic fungi in all but one of the samples analyzed. Moreover, besides this value (MRV) the need for a more demanding value was already stated in Sabino and colleagues (2011) [3] and the authors of this paper believe an adjustment to a non-coastal beach context is also required, based on extensive data which needs yet to be generated.

Special care needs to be paid to identify potential pathogenic fungal species, such as species from *Fusarium* (isolated in samples from recreational parks and in all elementary schools), *Aspergillus* genera (isolated in all schools analyzed besides one kindergarten) and *Neoscytalidium* genera (isolated from two schools and one kindergarten). In agreement with Rippon (1982) [14], sand may be considered to be a direct exposure source of geophilic, zoophilic and antropophilic keratinophilic fungi. Some of the isolated species in this study were previously detected in human and animal infections, such as *Aspergillus* spp., *F. solani* complex [15] and *Scopulariopsis* sp. [16]. *Scopulariopsis* species are known to induce opportunistic infections, and *S. brevicaulis* is well-established agent for onychomycosis [16]. This supports the notion of potential risk of skin/nail infections in children due to exposure to sand [17]. *Fusarium* genera are frequently implicated in ophthalmic keratitis and other superficial mycosis, and their presence in the analyzed sands is also an issue of concern for children health [12].

The existence of superficial infections caused by non-dermathophytic fungi have been reported in several studies [18-19]. When infections due to these other fungal agents occur, neglecting can lead to very unfavourable and even serious outcomes, such as opportunistic follow-up infections by bacteria [20]. Moreover, allergic reactions due to fungal exposure, especially due to dematiaceous fungi as *Cladosporium* spp. are well documented in several studies [21].

Conclusion

The fungal load found in the sand of some of the playgrounds analyzed in this pilot study suggests that sand has not been replaced nor treated (cleansed). The high fungal loads represent a potential human health risk and therefore, sandpits in such condition need to handle. The fungal species isolated also confirm the potential risk of exposure of children of young ages to keratinophilic fungi. Further studies applying molecular tools are needed to overcome eventual limitations from the methods applied. Despite the absence of epidemiologic studies to reaffirm concerns, this pilot study demonstrates that regular cleaning or replacing of sand needs to be implemented.

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