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ORIGINAL ARTICLE

Sandpits as a reservoir of potentially pathogenic fungi for children

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Abstract

Introduction and objective. Fungi belonging to various physiological and morphological groups present in the environment are potential human pathogens. Some of them are considered as emerging pathogens. Therefore, their presence in children's playgrounds should be regarded as health risk factor.

Materials and method. Sixty-eight samples of sand collected from 17 sandpits of different localities in Łódź, Poland, in autumn 2010 and 2011, and in spring 2011 and 2012 were evaluated. The fungi were isolated with classical mycological methods and identified on the basis of morphological and biochemical features.

Results. The prevalence of fungi in spring was 94.1% of sandpits in both layers of sand (depth 0–3 cm and 10–15 cm) and in one kindergarten sandpit, but only in a deeper layer. In autumn, fungi occurred in both layers in all sandpits (100%). The fungal concentration (CFU/g of sand) varied considerably (range 0 – uncountable) in both layers. A total of 352 isolates belonging to 80 species were found. There were 69 yeasts and yeast-like fungi isolates from 12 species (9 species in each season), and 283 filamentous fungi from 68 species: 35 species in spring and 55 in autumn, with 4 keratinolytic species. There were important causes of allergies, among them *Cladosporium herbarum* and *Alternaria alternata*, as well as of opportunistic mycoses: *Cryptococcus neoformans*, *Aspergillus fumigatus* and new and 'emerging' fungal pathogens e.g., *Trichosporon*, *Rhodotorula*, *Fusarium* and *Scedosporium* species.

Conclusions. Potentially pathogenic fungi are present in the sand taken from sandpits in Łódź. This fact poses a significant threat to child health and therefore proper maintenance and periodic checking of sandpits are of great importance.

Key words
potentially pathogenic fungi, health hazards, biological contamination of sandpits

INTRODUCTION

Fungi, as widely-dispersed microorganisms, may be detected in soil of areas of various human activities. There are filamentous and unicellular fungus forms with different distribution and different functions [1, 2]. Yeasts, yeast-like fungi and keratinophilic fungi take part in the mineralization of organic material, and interact with biotic factors, bacteria, other fungi, plants and animals. Most of the fungi are also potentially pathogenic to humans and animals. Children come into direct contact with soil when staying outside in the fresh air, often in sandpits, which can provide great playing and learning opportunities, but can also pose health and safety risks because of the presence of potentially harmful microorganisms, including fungi. Although several mycological evaluations of children's recreation places have been carried out, they have mainly considered fungi with affinity to keratin substrates, and/or were mostly performed in countries of southern Europe or in subtropical zones [3, 4, 5]. To-date, no such studies have been carried out in Poland. Therefore, the aim of the presented study was to determine the prevalence of potentially pathogenic fungi as a health risk for the local population in selected sandpits of the city of Łódź in Poland.

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MATERIALS AND METHOD

The surveys were carried out twice in autumn (October-November) 2010 and 2011, and twice in spring (April-May) 2011 and 2012 in Łódź, the third-largest city in Poland, characterized by a mean annual air temperature of 7.5 °C and relative air humidity of 80%.

Sand samples were collected from 17 localities in 5 districts of Łódź (Bałuty, Górna, Polesie, Śródmieście and Widzew): 5 from sandpits situated in kindergartens, 4 from sandpits of primary and secondary school playgrounds, 6 from sandpits located in housing estates of the city, and 2 from sandpits of the Źródliska Park (area of 17.2 ha) and the 3rd-May Park (23.5 ha) playgrounds. All examined kindergarten sandpits were situated within fenced areas; one was covered, when not used, to protect against animal soiling. Four sandpits of housing estates were surrounded by metal fencing not exceeding 1m in height with a 1m-wide gate, one was surrounded by a hedgerow, and one was not fenced directly, but located on a fenced playground. The sandpits of school playgrounds and public parks were located within fenced areas.

A total of 68 samples were collected: 24 samples during autumn 2010 and spring 2011 from the same 6 localities, and 44 samples during autumn 2011 and spring 2012 from the same 11 localities. Each sample of sand was taken from an area of about 5 m^2 at 6 various points (subsamples). The subsamples were combined into one composite sample of about 300 g. The samples were taken both from the 0–3 cm superficial layer and the depth of 10–15 cm from each examined site. Each sand sample was placed separately into a sterile plastic bag, which was labelled by a number and description, and stored at 4°C.

Isolation and identification of fungi. Isolation and identification of the fungi were performed as described in a previous study [6]. Fungal colonies grown in the Dichloran Rose Bengal Chloramphenicol (DRBC) Agar (Merck) were counted after 5 days of incubation, checked under a microscope and transferred onto Sabouraud Dextrose Agar to obtain the axenic cultural growth, and also onto Potato Dextrose Agar in the case of filamentous fungi. Mycological identification was based on both macroscopic and microscopic (using lactophenol blue staining) morphological observations, using identification atlases, as well as, for yeast-like fungi, on biochemical features [6, 7]. For keratinophilic fungi, the hair baiting technique of Vanbreuseghem was used. Approximately 50 g of each sand sample was placed into a sterile Petri dish, baited with sterilized 1–2 cm long strands of human child hair and moistened with 5–10 cm^3 sterile distilled water. The plates were incubated for up to 6 weeks in the dark, at room temperature, with weekly examination for mycelium development and/or macroconidia formation. Fragments of colonized hair were inoculated onto Sabouraud Dextrose Agar with cyclohexamide and chloramphenicol for morphological evaluation and identification of culture using standard procedures [7].

Statistical methods. The data was analysed using STATISTICA v.10.0 software. Mean values of parameters were compared between seasons and depth layers using ANOVA. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

A part of the results concerning yeasts and yeast-like fungi in sandpits evaluated in autumn 2010 and spring 2011 were published subsequently (2013) [6].

The mycological analysis of sand collected from 17 sandpits in Łódź revealed the presence of fungi in all of them (Tab. 1). In spring, in 16 sandpits (94.1%), fungi were found in both layers of sand and in one sandpit located in kindergarten area only in deeper layer. In autumn, fungi occurred in both layers in all sandpits (100%). Considering the number of colony-forming units (CFU) per g of sand in spring, the mean value for the superficial layer was 463.9 CFU/g (range 0–1440), and in the deeper layer, the range was of 0 – uncountable. In the autumn's evaluations, the mean values were 2233.4 and 2485.8 CFU/g (ranges 330–7980 and 201–14560 for the 2 depths, respectively). There was no statistically significant difference between the mean values for autumn ($p>0.5$) and for mean values for the depth of 0–3 cm during the 2 seasons ($p>0.9$).

The presence of yeasts and yeast-like fungi was noted in all sandpits, but in spring only in 13 (76.5%) and in autumn in 16 (94.1%) out of 17 evaluated (Tab. 1). In several cases, the fungi were present only in one of the evaluated layers: in spring, only in the surface layer in 2 sandpits, only in the deeper layer in 4, and in both layers in 7 (41.2%) sandpits; in autumn, only in the surface layer in 4, only in deeper in 3, and in both in 9 (52.9%). The mean values of CFU/g of sand were: in spring – 21.2 for the superficial layer (range 0–87), the mean value for the deeper layer was not calculated for uncountable growth in one sample (range 0-∞); in autumn – 72.6 for the superficial layer (range 0–293), and 53.7 for the deeper layer (range 0–193). There were no statistically significant differences in the mean values ($p>0.6$).

A total of 352 isolates belonging to 80 species were found: in spring 137 isolates from 44 species, and in autumn 215 from 64 species. There were 69 yeasts and yeast-like fungi isolates from 12 species (9 species in each season) and 283 filamentous fungi from 68 species: 35 species in spring and 55 in autumn (Tab. 2, Tab. 3). The total number of identified

Table 1. Total number of fungi (CFU/g of sand) and species number in samples from sandpits in Łódź

Sandpit localization (no. of sites sampled)	Depth/ season	Mean number of fungi x102 CFU/g of examined sand samples (range)				Mean isolate number of species recognized (range)			
		Total		Yeasts and yeast-like fungi		Total		Yeasts and yeast-like fungi	
		A	B	A	B	A	B	A	B
Kindergartens (5)	spring	465.2	*	28.0	*	2.60	2.40	0.80	1.00
		(0-1406)	(60-∞)	(0-60)	(0-∞)	(0-4)	(1-5)	(0-2)	(0-2)
	autumn	1316.8	4052.6	37.4	36.0	4.8	4.8	1.00	0.80
		(333-2720)	(206-14560)	(0-67)	(0-80)	(2-9)	(4-7)	(0-2)	(0-1)
Schools (4)	spring	220.4	798.0	28.3	42.3	4.75	4.25	0.75	1.75
		(70-605)	(68-1940)	(0 - 73)	(20-60)	(2-7)	(1-8)	(0-2)	(1-3)
	autumn	3373.3	317.8	18.0	18.4	6.75	6.5	0.75	0.75
		(526-7980)	(201-2040)	(0-66)	(0-53)	(3-12)	(3-11)	(0-2)	(0-2)
Parks (2)	spring	745.0	623.0	3.5	0	6.00	4.00	0.5	0
		(330-1160)	(366-880)	(0-7)	(0)	(5-7)	(4)	(0-1)	(0)
	autumn	2407.0	1780.0	73.5	76.5	8.50	6.50	1.00	1.50
		(894-3920)	(1740-1820)	(60-87)	(66-87)	(6-11)	(6-7)	(1-1)	(1-2)
Housing estates (6)	spring	531.7	376.2	13.3	36.8	3.33	4.17	0.67	0.83
		(120-1440)	(147-1180)	(0-73)	(0-60)	(1-6)	(1-7)	(0-2)	(0-2)
	autumn	2083.3	2527.3	138	84.5	8.50	6.00	2.00	1.33
		(1110-5420)	(820-4960)	(0-293)	(0-193)	(3-12)	(3-9)	(1-4)	(0-3)

A – depth 0-3 cm
B – depth 10-15 cm
* –average evaluation not possible

Table 2. Yeasts and yeast-like fungi frequency occurrence in sandpits of different town area in the two seasons

Isolated species	Number of isolates										Sandpits with the species	
	kindergartens		schools		parks		housing estates		total (%)			
	s	aut	s	aut	s	aut	s	aut	s	aut	No.	%
Candida famata	0	0	0	0	0	0	0	1	0	1	1	5.88
Candida guilliermondii	0	0	2	0	0	0	0	0	2	0	1	5.88
Candida lusitaniae	0	0	0	0	1	0	2	0	3	0	2	11.8
Cryptococcus albidus	1	1	1	0	0	0	0	1	2	2	3	17.6
Cryptococcus laurentii	0	1	2	1	0	1	0	3	2	6	6	35.3
Cryptococcus neoformans	3	1	1	1	0	1	2	1	6	4	6	35.3
Kloeckera japonica	1	0	0	0	0	0	0	0	1	0	1	5.88
Geotrichum candidum	1	0	1	1	0	1	0	2	2	4	5	29.4
Geotrichum penicillatum	0	2	0	0	0	0	0	0	0	2	2	11.8
Rhodotorula mucilaginosa	0	0	0	0	0	0	0	1	0	1	1	5.88
Rhodotorula glutinis	2	0	1	1	0	1	0	4	3	6	6	35.3
Trichosporon cutaneum	3	2	2	2	0	1	5	7	10	12	11	64.7
Total No. of isolates	**11**	**7**	**10**	**6**	**1**	**5**	**9**	**20**	**31**	**38**		

s – spring
aut – autumn

Table 3. Filamentous fungi frequency occurrence in sandpits of different city areas in the two seasons.

Isolated species	No. of isolates										Sandpits with the species	
	kindergartens		schools		parks		housing estates		total (%)			
	s	aut	s	aut	s	aut	s	aut	s	aut	No.	%
Keratinophilic fungi												
Acremonium alabamense	0	0	0	0	0	0	0	1	0	1	1	5.88
Acremonium atrogriseum	0	0	0	0	0	0	0	1	0	1	1	5.88
Acremonium falciforme	0	0	0	0	0	0	0	1	0	1	1	5.88
Acremonium hyalinum	0	1	0	0	0	0	0	0	0	1	1	5.88
Acremonium kiliense	5	0	1	0	0	0	0	0	6	0	3	17.6
Acremonium strictum	2	3	0	2	0	0	0	4	2	9	6	35.3
Alternaria alternata	1	3	2	3	3	1	0	4	6	11	10	58.8
Alternaria chlamydospora	0	0	0	2	1	0	0	0	1	2	3	17.6
Arthrinium phaeospermum	0	0	0	0	1	0	0	2	1	2	3	17.6
Arthrographis kalvae	0	0	1	0	0	0	1	0	2	0	2	11.8
Aspergillus fumigatus	0	0	0	0	0	0	0	1	0	1	1	5.88
Aspergillus ochraceus	2	0	0	0	0	0	1	0	3	0	2	11.8
Aureobasidium pullulans	0	0	0	1	0	0	0	1	0	2	2	11.8
Chaetomium sp.	0	1	0	1	0	0	1	0	1	2	3	17.6
Chrysosporium keratinophilum	0	2	0	3	0	0	1	2	1	7	5	29.4
Chrysosporium tropicum	0	1	1	0	0	1	0	2	1	4	5	29.4
Chrysosporium zonatum	0	0	0	1	0	0	0	0	0	1	1	5.88
Cladophialophora carrionii	0	1	0	0	0	1	0	0	0	2	2	11.8
Cladorrhinum bulbillosum	0	0	0	0	1	0	2	0	3	0	3	17.6
Cladosporium herbarum	5	7	3	5	3	1	3	5	14	18	16	93.1
Cladosporium sphaerospermum	0	0	0	1	0	3	0	1	0	5	4	23.5
Doratomyces stemonitis	0	0	0	0	0	1	0	0	0	1	1	5.88
Fusarium aquaeductum	0	0	0	0	0	0	0	1	0	1	1	5.88
Fusarium dimerum	0	0	0	0	0	0	0	1	0	1	1	5.88
Fusarium verticillioides	0	0	0	0	0	0	0	1	0	1	1	5.88
Fusarium nygamai	0	0	0	0	1	0	0	0	1	0	1	5.88
Fusarium oxysporum	2	2	3	5	2	0	3	2	10	9	9	52.9

Table 3. Filamentous fungi frequency occurrence in sandpits of different city areas in the two seasons (continuation)

Isolated species	No. of isolates										Sandpits with the species	
	kindergartens		schools		parks		housing estates		total (%)			
	s	aut	s	aut	s	aut	s	aut	s	aut	No.	%
Fusarium poae	0	0	0	1	0	0	0	0	0	1	1	5.88
Fusarium solani	1	1	0	1	0	0	5	2	6	4	7	41.2
Hormographiella sp.	0	0	0	2	0	0	0	0	0	2	1	5.88
Humicola fuscoatra	0	0	0	0	0	0	0	1	0	1	1	5.88
Humicola grisea	0	0	0	1	0	0	0	0	0	1	1	5.88
Lecythophora mutabilis	0	0	1	0	0	0	0	0	1	0	1	5.88
Mucor circinelloides	0	0	1	0	0	0	0	0	1	0	1	5.88
Mucor hiemalis	0	0	0	1	2	1	3	3	5	5	7	41.2
Mucor racemosus	0	0	0	2	0	1	0	2	0	5	5	29.4
Mucor ramosissimus	0	0	0	1	0	1	0	2	0	4	4	23.5
Morterella polycephala	0	0	0	0	1	0	0	0	1	0	1	5.88
Paecilomyces lilacinus	2	1	0	2	0	0	3	0	5	3	6	35.3
Paecilomyces niveus	0	0	0	0	0	1	1	0	1	1	2	11.8
Paecilomyces variotii	0	2	1	0	1	2	2	3	4	7	7	41.2
Paecilomyces viridis	0	0	0	0	0	0	0	1	0	1	1	5.88
Penicillum chrysogenum	0	4	3	1	1	2	5	5	9	12	13	76.5
Penicillium commune	0	2	0	0	0	0	0	1	0	3	2	11.8
Penicillium decumbens	0	0	0	0	0	0	0	1	0	1	1	5.88
Penicillum expansum	0	1	0	1	2	0	0	0	2	2	3	17.6
Penicillium rugulosum	0	0	0	0	0	0	0	4	0	4	3	17.6
Penicillium terrestre	0	0	2	0	0	0	0	0	2	0	1	5.88
Penicillium spinulosum	0	0	0	0	0	0	0	1	0	1	1	5.88
Penicillium verruculosum	0	0	0	0	0	1	0	1	0	2	2	11.8
Penicillium waksmanii	0	0	0	0	0	0	0	1	0	1	1	5.88
Rhizomucor pusillus	0	0	0	0	0	0	0	1	0	1	1	5.88
Rhizomucor variabilis	0	0	0	0	0	1	1	1	1	2	3	17.6
Scedosporium apiospermum	0	0	0	1	0	0	0	0	0	1	1	5.88
Scedosporium prolificans	0	0	0	0	0	0	0	1	0	1	1	5.88
Scopulariopsis acremonium	0	0	1	0	0	0	0	0	1	0	1	5.88
Scopulariopsis sp.	0	0	1	0	0	1	0	0	1	1	2	11.8
Scytalidium infestans	0	0	0	0	0	1	0	0	0	1	1	5.88
Scytalidium lignicola	0	1	0	0	0	0	0	2	0	3	3	17.6
Staphylotrichum coccosporum	0	0	0	0	1	0	0	0	1	0	1	5.88
Trichoderma viride	1	1	1	0	1	2	2	4	5	7	8	47.1
Trichoderma harzianium	0	0	0	0	0	1	0	0	0	1	1	5.88
Verticillium sp.	0	3	0	3	0	2	1	1	1	9	8	47.1
Volutella cinerescens	0	0	0	0	0	1	0	0	0	1	1	5.88
Total No. of isolates	**21**	**37**	**22**	**41**	**21**	**26**	**35**	**68**	**99**	**172**		
Keratinolytic fungi												
Keratinomyces ceretanicus	1	0	0	0	0	0	0	0	1	0	1	5.88
Microsporum gypseum	0	1	2	3	0	0	0	0	2	4	3	17.6
Trichophyton ajelloi	0	0	2	1	0	0	1	0	3	1	3	17.6
Trichophyton terrestre	0	0	1	0	0	0	0	0	1	0	1	5.88
Total No. of isolates	**1**	**1**	**5**	**4**	**0**	**0**	**1**	**0**	**7**	**5**		

s – spring
aut – autumn

fungal species varied from 0 to 12 in different sand samples. The mean number of species in all sandpits was higher in autumn. Overall, in autumn there were 2–12 and 3–11 species identified, and in spring 0–7 and 1–8 for the depth of 0–3 cm and of 10–15 cm, respectively. There were no statistically significant differences in the mean values between seasons ($p>0.9$) and between the evaluated layers ($p>0.3$ for spring and $p>0.8$ for autumn). The mean number of yeasts and yeast-like fungi species was in the range of 0–2 and 0–3 in spring, and 0–4 and 0–3 in autumn for the 2 layers, respectively, with a lack of significant differences ($p>0.7$).

The greatest number of species from all evaluated seasons and layers was identified in one park and one school sandpit (25 in each), also from 2 sandpits of housing estates (21 and 17); the number of different species was lower (7–9) from the kindergarten sand samples. The most often widespread species were: *Cladosporium herbarum* found in 16 sandpits, *Penicillium chrysogenum* in 13, *Alternaria alternata* in 10, *Fusarium oxysporum* in 9, *Trichoderma viride* in 8; and yeast-like fungi: *Trichosporon cutaneum* in 11, *Cryptococcus laurentii* in 6, *Cryptococcus neoformans* in 6, and *Rhodotorula glutinis* in 6.

Using the hair baiting technique, 4 keratinophilic species were recognized almost only in the superficial layers of the examined localities (Tab. 3). *Keratinomyces ceretanicus* was present only in one kindergarten sandpit and *Trichophyton terrestre* was found in the sample from one school sandpit, each in spring. The species *Microsporum gypseum* and *Trichophyton ajelloi* were identified, both in the 2 seasons, in 2 school playground sandpits. Moreover, *M. gypseum* was found in one kindergarten sandpit and *T. ajelloi* in one of sandpits located in housing estate.

The data revealed in the presented study demonstrate a large range of values. The greatest variability of all fungi CFU/g of sand and also yeasts and yeast-like fungi CFU/g of sand was observed in kindergarten sandpits. In one case, 61 *C. neoformans* colonies/g of sand with the lack of other fungal colonies were stated. In another kindergarten, in spring, the uncountable growth of *Geotrichum candidum* with single *Cryptococcus albidus* colonies was observed. Three *Candida* species were found, each in a single sandpit and with low concentration values within the range of 17–33 CFU/g (Tab. 2). Of note, in the kindergarten sandpit which was covered when not used, to protect against animal soiling, varying fungal concentrations were detected: in spring 146 and 940 CFU/g, and in autumn 2,720 and 14,560 CFU/g for the superficial and deeper layer, respectively.

DISCUSSION

The results of the presented study showed considerably varying fungal concentrations and species distribution in the sand of sandpits from different city localities in the observed seasons. The total number of CFU/g of sand was within the range of 0–14560. This may suggest that the sand was exchanged in some of the evaluated sandpits during the period of investigations. The Chief Sanitary Inspectorate in Poland, for proper maintenance of sandpits, has recommended the owners or administrators of children's playgrounds the total exchange of the sandpit sand once before a season, and at least once during a year of its use. Moreover, fencing and/or covering sandboxes in order to prevent cats, dogs and birds entering play areas is also very important and recommended. The Regional State Sanitary-Epidemiological Stations evaluate sanitary conditions of selected sandpits only for the presence of parasitic geohelminths. However, in the majority of cases, the exchange of sand is in reality recommended on the basis of visual evaluation.

The fungi present in soil belong to various taxonomic groups and differ morphologically and physiologically. Some of them show affinity to keratin substrates, including keratinophilic fungi which naturally colonize such substrates, and keratinolytic ones (dermatophytes) capable of attacking and demolishing keratin [1]. In this study, keratinophilic fungi were predominant with the highest frequency of species of genera: *Penicillium*, *Fusarium*, *Cladosporium*, *Alternaria*, *Mucor*, *Paecilomyces* and *Acremonium*.

In the presented study, the higher total concentration and a greater number of fungal species were observed in the samples collected in autumn; however, this did not concern yeast-like fungi. The observations concerning keratinolytic and keratinophilic species of children`s sandpits in Turin, Italy, showed their appreciably greater numbers isolated in June, October and December than in March, but the sandpits were evaluated only once in a chosen season [3]. The highest frequency of occurrence among the Turin sandpits concerned fungi of the genera: *Alternaria*, *Cladosporium*, and *Paecilomyces*, which were also very frequent in the presented evaluations. In the Turin studies, a very high prevalence of *Aphanoascus*, *Trichophyton*, *Chrysosporium*, *Gliocladium* and *Geomyces* was also identified.

The keratinophilic fungi in the current study were found in the both evaluated sand layers, while dermatophytes were detected almost only in the superficial layer. Other studies have demonstrated that fungi are present in the greatest number in soils with a high humus content and in the greatest amount in the superficial layers (up to 25 cm) [8]. The main factor favouring the occurrence of keratinolytic and keratinophilic fungi in soil is the presence of keratinous debris. Anthropogenic habitats, such as sandpits in children`s play areas, are places of possible input of keratin. Fungal structures may be introduced into sand with epithelial scales or falling hair. Screening of pupils aged 6–15 years in India for scalp fungi revealed a great spectrum not only of dermatophytes, but also of other keratinophilic fungi [9]. The presence of fungi in sandy soils is related to their tolerance and adaptation to various abiotic and biotic factors. It is worth mentioning that some keratinophilic strains remain in extreme environment conditions – e.g. cold desert in the Himalayan region or a region with high temperature and humidity [10, 11].

The species *T. ajelloi* and *M. gypseum* detected in 4 of the Łódź sandpits are considered as geophilic dermatophytes with a worldwide distribution. In Turin, they were recognized in 60.7% and 10.7% of sandpits, respectively [3]. They were also shown in the soils, stressed by the presence of animals, both pet dogs and cats, as well as farm animals [12]. In sandpits of the Nablus areas of the West Bank of Jordan, dermatophytes: *M. gypseum* (17.2%), *Trichophyton mentagrophytes* (6.9%) and *Microsporum audouinii* (3.5% of sandpits) were detected [4]. In the Łódź sandpits, there were neither dermatophytes, such as *Microsporum canis* or *T. mentagrophytes,* nor other dermatophytes responsible for human skin mycoses. The mentioned species were the most commonly isolated from dogs and cats [13, 14]. Only *M. gypseum* species was the cause of 5.3% of tinea cutis glabrae cases in Poland [15]. *Trichophyton ajelloi* may occur as a saprobiont contaminant on humans and animals, but very rarely causing infections [14].

Interestingly enough, birds may also introduce fungi into soil and the sand of sandpits. The most frequent genera in the bird plumage in the Czech Republic and former Yugoslavia were: *Alternaria*, *Cladosporium, Arthroderma*, *Aspergillus*, *Penicillium*, *Chrysosporium*, and also *Mucor*, *Rhizopus, Fusarium*, *Paecilomyces* [16]. Epidemiological evaluations of feral pigeon (*Columba livia*), very popular birds in the city of Łódź, showed that 45 potential human fungal pathogens were harboured by them [17]. Some of the pathogens mentioned in the review were also found in the Łódź sandpits, such as: *C. guillermondii*, *C. lusitaniae*, *C. laurentii*, *C. albidus*, *C. neoformans*, *G. candidum*, *R. glutinis*, *R. rubra* (syn. *R. mucilaginosa*), *T. cutaneum* and *Paeciliomyces* spp.

The species *C. neoformans*, most commonly affecting immunocompromised individuals, was present in the samples from the sandpits of 2 kindergartens, in one sample from a school and in 2 from the housing estates sandpits. The species *C. laurentii* was detected in 6 and *C. albidus* in 3 evaluated sandpits. It was proved that among all *Cryptococcus* species, environmental exposure only to *C. neoformans* can be harmful for the human organism, causing respiratory infection, as well as skin or life-threatening central nervous system mycoses.

Considering that there is very little information about fungi in sandpits in the available literature, an attempt was made to refer the presented results to other sandy places, for example, beaches. Sand from sea beaches, but not from rivers, is accepted for the use in sandpits in Poland. Analysis of sand from 33 Portugal beaches demonstrated 60.4% of positive samples within the range of 0–1934 CFU/g [18]. The yeast-like fungi were detected in 25.4% (67.5% identified as *Candida* species) and dermatophytes in 14.3% of samples (genera *Trichophyton* and *Microsporum*). Potential pathogenic fungi, other than those mentioned above, constituted 47.9%, with *Aspergillus* being the predominant genus, followed by *Fusarium*, *Scedosporium*, *Scytalidium* and *Chrysosporium*. The investigations of beaches in Korea showed 31.0% of positive samples for keratinophilic fungi [19]. The most frequent species were *Chrysosporium* strains (27.3% of samples); *M. gypseum* was recognized in 4.5% and *T. ajelloi* in 0.8% of samples. On the beaches of the Ligurian coast of Italy, *Cladosporium*, *Papulaspora*, *Penicillium*, *Microsporum* were the most widespread genera [20]. The highest percentage of total strains were represented by the genus *Acremonium*, but its distribution was limited to a single beach. In sand in subtropical beaches of the USA, yeast-like fungi, *Rhodotorula* and *Candida,* were recognized [21]. The species *C. tropicalis* showed the highest frequency (15 CFU/g), other pathogenic species were rare: *C. guiliermondii*, *C. glabrata* and *C. parapsilosis* (1.1, 1.3, 1.3 CFU/g, respectively).

Among the yeasts and yeast-like fungi in the presented study, the most prevalent was *T. cutaneum,* isolated from 64.7% of sandpits. *Trichosporon* spp. are widely distributed in nature and found predominantly in tropical and high temperate areas. It is worth considering, that the species *T. cutaneum* may be a part of the normal biota of the human skin, vagina, respiratory and gastrointestinal tract. But it may also cause superficial infections, predominantly in immunocompetent hosts, or induce invasive diseases in immunocompromised subjects [22].

A total of 35.3% of the sandpits evaluated for the current study were comprised of *R. glutinis* cells. Species of the *Rhodotorula* genus are ubiquitous saprobionts that can be recovered from many environmental sources. Found in 4–25% of the samples, *R. glutinis* was the most frequently isolated carotenoid-producing species from 320 samples of tree leaves and needles [23]. In Cádiz, Spain, the species was the most frequently isolated, both from different human ontocenoses (78.9%) and samples of water and pigeon excreta (92.4% of the strains) [24]. Previously considered non-pathogenic, the *Rhodotorula* species as well as *R. glutinis,* have emerged as opportunistic pathogens that have the ability to colonise and infect humans [25].

Geotrichum candidum, found in the 5 evaluated sandpits (in one with uncountable concentration) is a ubiquitous fungus which is rarely pathogenic to humans. It can colonize the human gastrointestinal and respiratory tracts. This species is widely used as adjunct culture in the maturation of cheese, but due to its technological use or consumption of dairy products it does not cause infection [26].

The spores and metabolites of the soil-born fungi may persist in air as part of bioaerosol causing allergies (aeroallergens). The peak of mould spore concentration is mostly noticeable in the air in late summer and early autumn [27]. A great number of isolates of different mould species have been observed, the number of which rose in the sand in autumn. This may also be a factor greatly affecting the health of children playing in outdoor areas.

It must be stressed that almost all fungal species identified in the Łódź sandpits are now regarded as emerging human pathogens with significant resistance to standard antifungal therapy [28, 29]. The fungi species: *A. kiliense*, *F. oxysporum*, *F. solani*, *P. lilacinus*, *S. apiospermum* usually inhabiting the natural environment can cause human mycoses, the frequency of which has increased significantly over the past 2 decades.

The presented mycological evaluation of sand from 17 sandpits of playgrounds in Łódź highlights potentially significant threats to children`s health. In Poland, legal Acts are to guarantee the safe and healthy play environment for children. Moreover, the playground equipment and some safety aspects come under the terms of The General Product Safety Directive 2001/95/EC (The European Standards for Playground Equipment: EN 1176 and EN 1177). However, the proper maintenance and periodic checking of sand in sandpits and all playground areas is still a big problem. Unfortunately, it is sometimes easier to close down sandpits than follow the law. Nowadays, the data of the Ombudsperson for the Children of Poland show that 10% of 400 kindergartens and 77% of school playgrounds do not possess any sandpits.

CONCLUSIONS

Potentially pathogenic fungi are present in the sand taken from sandpits in Łódź. This fact poses a significant threat to children's health, and therefore proper maintenance and periodic checking of sandpits are of great importance.

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