Science of the Total Environment 470–471 (2014) 646–650

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short Communication

Snow in the city as a spore bank of potentially pathogenic fungi

Elżbieta Ejdys *[,1], Anna Biedunkiewicz, Maria Dynowska, Ewa Sucharzewska

Department of Mycology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 1A, 10-957 Olsztyn-Kortowo, Poland

HIGHLIGHTS

- The analysis of snow cover as an ecological niche and vector especially for fungal pathogens
- Comparison tests of the air, snow and ice.
- The analyzed samples of snow cover contained from 101.6 to 8500.0 CFU/m^3 of fungi
- In the samples of snow and ice most of the isolates were anamorphic (asexual), in the air only sexual.
- 26 species of yeast and yeast-like fungi were isolated from the experimental material (10 from snow).

ARTICLE INFO

Article history:
Received 14 May 2013
Received in revised form 13 October 2013
Accepted 14 October 2013
Available online 28 October 2013

Keywords:
Snow
Fungi
City
Atmospheric air

ABSTRACT

This study evaluates the role of snow as a specific ecological niche and a vector in fungal spreading with particular emphasis on potential pathogens in seasonally and daily changing conditions.
The experimental material was fungi isolated from the atmospheric air, snow cover, and fragments of ice and soil from underneath the snow cover. The total count of microfungi in the air before snowfall, i.e. in the autumn, reached 1756.1 CFU/m^3 on average. After the first snowfalls, it dropped to 85.2 CFU/m^3. The analyzed samples of snow cover contained from 101.6 to 8500.0 CFU/m^3 of fungi. Furthermore, 26 species of yeast and yeast-like fungi were isolated from the experimental material. Amongst the analyzed species, 13 were potential anthropopathogens. Though another three species were isolated from organ ontocenoses, i.e. *Candida intermedia*, *Saccharomyces bayanus* and *Zygosaccharomyces rouxii*, their pathogenic potential has not yet been explicitly confirmed. The results of the presented study may be applied in predicting concentrations of fungal spores responsible for mycoses. The first snowfalls significantly reduced the number of colony-forming units of fungi in the air. Under conditions of temperate climate, snow becomes a temporary bank of yeast-like fungi spores and while it melts cells of deposited microfungi migrate to the atmosphere. Hence, individuals with impaired immunity or in the course of immunosuppression or recovery should avoid long walks during periods of snow melting. The count of fungi in urban bioaerosol during the melt may be reduced through systematic removal of snow cover, which is a significant reservoir of potential pathogens. In addition, it should be noted that even a typical psychrophilic strain, capable of surviving at a temperature of 37 °C, may bear a significant pathogenic potential.

1. Introduction

One of the major elements used in environment diagnosis is the evaluation of its degradation in the context of the epidemic hazard posed by microfungi and vectors carrying them (Dynowska and Kisicka, 2005; Shen and Yao, 2013). Air is a crucial vector for fungal spores deposited mainly in the soil which are important components of aeroplankton. Fungi and their metabolites (very often with high toxicity) penetrate into an organism through airways, the digestive system and the skin. The concentration of fungal spores in the air depends on the size and type of habitat as well as on the variability of its parameters.

The concentration of fungal spores in the atmospheric air of urban environments varies from their concentration in non-urban areas both in the aspects of daily and seasonal fluctuations (Kasprzyk, 2006). This is connected with the specific "urban microclimate" regulated, amongst other things, by urban breezes which carry the fungal spores from extra-urban sources. For this reason, the concentration of the spores in urbicenoses is normally higher than in natural ecosystems. In addition to the typical saprotrophs and phytopathogens (Biedunkiewicz, 2007a, 2009; Biedunkiewicz and Baranowska, 2011; Kasprzyk et al., 2013; Sucharzewska et al., 2012), there are fungi which are potentially pathogenic to humans constantly present in various urban environments (Dynowska, 1995; Ejdys et al., 2009; Pei-Chih et al., 2000). Theoretically, greater agglomerations will result in more people suffering from mycoses of different etiology and there will be more fungi carriers emitting fungal cells to the atmosphere.

* Corresponding author.
E-mail address: elzbieta.ejdys@uwm.edu.pl (E. Ejdys).
[1] Tel./fax: +48 89 523 42 95.

0048-9697/$ – see front matter
http://dx.doi.org/10.1016/j.scitotenv.2013.10.045

The count of microfungi in the air is regulated by precipitation (Bauer et al., 2010; Kasprzyk, 2006; Liao et al., 2004). Rainfalls form around the condensation nuclei which can be in a gaseous, liquid or solid state, whereas snow can form only if the crystallization nuclei are in a solid state. It is of no importance, however, whether they have abiotic (dust) or biotic (living organisms or their fragments) characteristics. Throughout the entire vegetative season, especially in the autumn, spores and mycelial fragments may constitute the greatest part of the dispersed phase of outdoor bioaerosol and constitute the majority of the crystallization nuclei of solid precipitation, particularly snow. Almost every year, the snow cover in most parts of Poland lasts at least from 5 to 10 days with a frequency varying from 70% to 90%. In the eastern parts of the country, however, the snow cover lasts for much longer — up to 30 days, while in unusually snowy years it lasts for over 60 days (Czarnecka, 2011).

The objective of this study was to evaluate the role of snow as a specific ecological niche and a vector in fungal spreading, with particular emphasis on potential pathogens in seasonal and daily changing conditions.

2. Material and methods

The experimental material was fungi isolated from the atmospheric air, snow cover, and fragments of ice and soil from underneath the snow cover from November 2010 till May 2011. The research stations (n = 12) were located 5 m away from the *Collegium Biologiae* building (at the Kortowo university campus in Olsztyn), alongside building walls (Table 1). Samples were collected in triplicate and, at each location, air was sampled with the Koch sedimentation method (on Sabouraud agar, Czapek-Dox medium and rose Bengal) and snow samples were taken from the snow cover and fragments of ice using the authors' own method according to the original, elaborated procedure presented in Fig. 1 (Biedunkiewicz, 2011). Soil samples were collected from beneath the snow cover at a depth of 10 cm. The qualitative and quantitative evaluation of fungi from soil samples was conducted with the plate method on Sabouraud agar with antibiotics and Czapek-Dox agar. Fungal cultures were incubated at 25 °C and 37 °C.

The total number of colony-forming units (CFU) of fungi was determined in the air (according to the Polish Standard, 1989) and in the snow per 1 m^3. In the case of snow and ice, these values were expressed in 1 dm^3 of snow water and ice water.

Pathogenic fungi were identified according to a routine diagnostic procedure (Fig. 2 — Dynowska et al., 2004). Yeast and yeast-like fungi identification was based on keys by Kurtzman et al. (2011).

3. Results

The total count of microfungi in the air before snowfall, i.e. in the autumn, reached 1756.1 CFU/m^3 on average (Table 1). After the first snowfalls, it dropped to 85.2 CFU/m^3. In the springtime, the number of microfungi in the atmospheric air was at 1847.9 CFU/m^3 on average. The highest number of fungal spores in bioaerosol was usually recorded at southern stations.

The analyzed samples of snow cover contained from 101.6 to 8500.0 CFU/m^3 of fungi. In snow water and ice water, the number of colony-forming units was within the range from 2.0 to 978 per 1 dm^3 for snow, and from 2300 to 11,385 per 1 dm^3 for ice.

Furthermore, 26 species of yeast and yeast-like fungi were isolated from the experimental material (Table 2). In the samples of air, the isolates were only teleomorphic (sexual), whereas most of the isolates in the samples of snow and ice were anamorphic (asexual). In the samples containing *Cylindrocarpon* — *C. lichenicola* and *C. cyanescens*, the growth of other fungi species was inhibited. No microfungi were detected in the soil samples from underneath the snow cover.

4. Discussion

The concentration of fungi in urbicenosis is determined, to the greatest extent, by the geographic location, urban development plan and the area of the green belt. The greatest prevalence of yeast-like fungi has been found in city centers and in urban green oases, e.g. in botanical gardens (Šegvić Klarić and Pepeljnjak, 2006). The analyzed campus meets both criteria. Two-thirds of the area of the 161-ha campus, located along a lake on the edge of a forest complex, includes an over 100-year-old park with green squares located close to university buildings. Such a vast species composition of yeast-like fungi in the air of the Kortowo campus is thus somewhat unsurprising. In addition, the campus is a place of work and education to 35,000 students and academic staff. They may be an important reservoir of pathogenic fungi, especially since these species were isolated in earlier studies from the inhabitants of Olsztyn at different ages and with various health statuses (Dynowska et al., 2001, 2002, 2006, 2008, 2011; Ejdys, 2006, 2008b; Biedunkiewicz, 2007b).

Amongst the analyzed species, 11 were potential anthropopathogens (Classification of organisms, 2004; Kurtzman et al., 2011). Though another four species were isolated from organ ontocenoses and blood, i.e. *Candida intermedia*, *Saccharomyces bayanus*, *Zygoascus hellenicus* and *Zygosaccharomyces rouxii* (Dynowska et al., 2011; Ejdys, 2008b; Kurtzman et al., 2011), their pathogenic potential has not been explicitly confirmed. It is worth emphasizing that the most significant human pathogens are, simultaneously, saprophytes which use a wide spectrum of nutrient substrates and tend to colonize habitats related to anthropogenic transformations of the environment, including food (Moreira et al., 2001), bottled water (Yamaguchi et al., 2007), construction materials (Ejdys, 2008a) and wastewaters (Biedunkiewicz and Ozimek, 2009). This results from the ecological flexibility of these fungi and their expansiveness in the colonization of new habitats. The species with undefined pathogenicity status may also include pathogenic strains. A good example in this case may be *Saccharomyces cerevisiae*. Some species of this common genus are probiotic (Büchl et al., 2010), whereas others are pathogenic (Dynowska et al., 2006).

Table 1
Total number of fungi in the air, snow and soil, depending on location.

Places/number of research stations	Air[a] before the first snowfall [CFU/m^3]/n = 54[b]	Snow cover I[c] [CFU/m^3]/n = 36[b]	Air[a] after snowfall [CFU/m^3]/n = 54[b]	Snow cover II[c] [CFU/m^3]/n = 36[b]	Air[a] in the spring [CFU/m^3]/n = 54[b]	Soil[d] [CFU/g]/n = 36[b]
N/3	969.9	53.3[e]	78.63	13. 3[e]	1389.2	0.0
E/3	1074.7	80.0[e]	26.21	326.6[e]	2804.6	0.0
S/3	3328.9	3260.0	235.9	60.0[e]	2149.4	0.0
W/3	1651.0	8500.0	0.0	6.6[e]	1048.5	0.0
ā		2973.3[e]		101.6[e]		
	1756.1	5880.0	85.2		1847.9	0.0

[a] Air samples = 3 media × 3 repetitions × 2 incubation temperature × 3 research stations = 54.
[b] In total for three research positions.
[c] Snow samples = 2 media × 3 repetitions × 2 incubation temperature × 3 research stations = 36.
[d] Soil samples = 2 test periods × 3 repetitions × 2 incubation temperature × 3 research stations = 36.
[e] In the samples were fungi of the genus *Cylindrocarpon*.