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Health hazards related to conidia of *Cladosporium*—biological air pollutants in Poland, central Europe

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ABSTRACT

The spores of *Cladosporium* Link. are often present in the air in high quantities and produce many allergenic proteins, which may lead to asthma. An aerobiological spore monitoring program can inform patients about the current spore concentration in air and help their physicians determine the spore dose that is harmful for a given individual. This makes it possible to develop optimized responses and propose personalized therapy for a particular sensitive patient. The aim of this study was to assess the extent of the human health hazard posed by the fungal genus *Cladosporium*. For the first time, we have determined the number of days on which air samples in Poland exceeded the concentrations linked to allergic responses of sensitive patients, according to thresholds established by three different groups (2800/3000/4000 spores per 1 m³ of the air). The survey was conducted over three consecutive growing seasons (April–September, 2010–2012) in three cities located in different climate zones of Poland (Poznan, Lublin and Rzeszow). The average number of days exceeding 2800 spores per cubic meter (the lowest threshold) ranged from 61 (2010) through 76 (2011) to 93 (2012), though there was significant variation between cities. In each year the highest concentration of spores in the air was detected in either Poznan or Lublin, both located on large plains with intensive agriculture. We have proposed that an effective, science-based software platform to support policy-making on air quality should incorporate biological air pollutant data, such as allergenic fungal spores and pollen grains.

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Introduction

Fungi of the genus *Cladosporium* Link. occur in different climatic zones as cosmopolitan, ubiquitous organisms. Genus *Cladosporium*, composed of numerous species, belongs to the family Davidiellaceae, order Capnodiales, class Dothideomycetes, and phylum Ascomycota (Index Fungorum). Dugan et al. (2004) reported 772 species names, and the current list of Index Fungorum (November 2016) contains 779 names, including 651 species and 128 varieties. They are secondary parasites and saprotrophs, widespread through the growing season. The fungi can inhabit living plant tissue such as stems, leaves, flowers and fruit (Ellis and Ellis, 1985; Marcinkowska, 2003; Horst, 2013; Lee and Liao, 2014), as well as dead stubble, hay and different composts (Lacey and Dutkiewicz, 1994). The fungi are commonly found on a variety of other substrates, such as rubber, insulation materials, stones, concrete, bricks and mortar, as detailed in the review by Gutarowska (2014). Moreover, *Cladosporium* readily grows on paper, and greatly contributes to the biodegradation of museum and library collections (Zielinska-Jankiewicz et al., 2008; Skóra et al., 2015). The substrates for fungal development include wood, textiles and wallpaper, so it is also responsible for the deterioration of the interiors of residential buildings (Andersen et al., 2011; Mandal and Brand, 2011). The mycelium of *Cladosporium* grows in refrigerators and on moist window frames (Barnes et al., 2001).

The spores of *Cladosporium* contribute to and often dominate in atmospheric bioaerosols (Elbert et al., 2007). According to Fröhlich-Nowoisky et al. (2009), concentrations of conidia of *Cladosporium* in the atmosphere over cultivated regions usually range between 1 and 10,000 spores per cubic meter of air. The spores of *Cladosporium* are biological air pollutants that pose a threat to human health. They irritate the respiratory tracts and contain numerous proteins that cause inhalant allergies in people (Resano et al., 1998; Kurup et al., 2002; Thompson et al., 2000; Rapiejko et al., 2004). Due to their small size (3–35 µm) the spores of *Cladosporium* can easily reach the lower respiratory tract, including the lungs (D'Amato and Spieksma, 1995; Reponen et al., 2001; Lee and Liao, 2014). The symptoms of inhalant allergies include allergic rhinitis, sinusitis and conjunctivitis, mold asthma and allergic alveolitis (Knutsen et al., 2012). It seems likely that the history of human exposure to these allergens will date back tens of thousands of years, since the spores of *Cladosporium* are abundant in the air inside of caves (Docampo et al., 2011). In Poland, the highest intensities of *Cladosporium*-associated allergic symptoms were recorded in summer and autumn (Lipiec et al., 2007). In museums and art conservatories, personnel are exposed to these fungi all year round; allergy to fungal spores, mainly consisting of *Cladosporium*, was observed in up to 85% of staff (Wiszniewska et al., 2010). The two most common species present in biological aerosol particles are *Cladosporium cladosporioides* and *Cladosporium herbarum* (Després et al., 2012).

Fungal allergens are proteins and glycoproteins with molecular weights usually between 6 and 90 kDa (Burge and Rogers, 2000). Thompson et al. (2000) documented two allergens of *C. cladosporioides* and nine allergens of *C. herbarum*. Additionally, the Allergome web site (www.allergome.org) lists six other

Cladosporium allergens. The Allergome database contains data on the allergenicity of allergens in their natural conformations and also for allergens obtained by means of molecular biology techniques, if available. There is also one *in silico* predicted protein from *Cladosporium malorum* Alt mr 1 (137aa) classified as an Alt a 1-related allergen. The total number of proteins identified as allergens in *Cladosporium* fully or partially characterized and listed in different international scientific journals and web databases is close to twenty, of which thirteen have been cloned.

While species of *Cladosporium* are capable of producing multiple allergens, it should be noted that not all may be expressed in conidia. According to Aukrust (1979), out of ten allergens found in the genus *Cladosporium*, eight were detected in *C. herbarum*, but only one of these ten allergens was detected in fungal conidia (Cl a h HCh-1); the remaining ones were found in mycelium. Dixit and Kwilinski (2000) reported allergenic cross-reactivity between *C. cladosporioides*, *C. herbarum* and *Cladosporium sphaerospermum*.

Volumetric traps frequently record *C. herbarum*-type spores, which occur mainly in temperate and cold climate zones (Hjelmroos, 1993). Concentrations and annual total sums of these spores depend on geographic area, the location of measuring devices and timing of the growing season (Grinn-Gofron et al., 2011; Aira et al., 2012). Thus, aerobiological monitoring may be a valuable tool to patients and physicians that helps to identify a harmful dose of allergenic spores.

Various authors have established different threshold values for the *Cladosporium* spore concentrations causing allergy symptoms in average sensitive patients. The threshold value to evoke the first allergy symptoms in humans is 80 to 100 spores per 1 m³ of air, as reported by Rapiejko et al. (2004) and Gravesen (1979), respectively. The spores of *Cladosporium* are small (4–25 × 3–7 µm), so high concentrations were postulated to induce allergenic symptoms. For severe asthma, Rantio-Lehtimäki et al. (1991) proposed the threshold of 4000 spores per 1 m³ of air, D'Amato and Spieksma (1995) reported 3000 spores and Rapiejko et al. (2004) cited a figure of 2800 spores per 1 m³. Rantio-Lehtimäki et al. (1991) based the threshold on the relation of the size of *Cladosporium* spores to the size of birch pollen grains (0.03). D'Amato and Spieksma (1995) established the threshold using a combination of the results of aerobiological studies, positive diagnostic tests and careful recording of symptoms elicited by the allergens produced by *Cladosporium*. The development of the threshold by Rapiejko et al. (2004) was based on studies conducted in the years 2002–2003, concerning the intensity of allergic rhinitis and bronchial asthma symptoms in 600 patients living and working in two districts of Warsaw, Poland. Patients were sensitive to the allergens of some pollen grains, as well as to the spores of *Cladosporium*. The studies took into account aerobiological measurements, patient self-observation sheets and medical examination, including endoscopy of nasal cavities assessing color, edema of the nasal turbinates and the presence and intensity of nasal congestion, as well as conjunctival symptoms.

The aim of this study was to assess the extent of the human health hazard posed by the fungal genus *Cladosporium* by determining the number of days on which the spore concentration threshold for allergic responses was exceeded.

The survey was conducted over three consecutive growing seasons (April–September, 2010–2012) in different climate zones of Poland (central Europe).

1. Experiment

1.1. Monitoring sites

The studies were performed over three consecutive years (2010–2012) from 1 April to 30 September. The survey was conducted in Poland at three locations, one in the western part (Poznan), which is representative of the intermediate-mild weather encountered in western Europe, and two in the east of the country (Lublin and Rzeszow), with a more harsh and continental climate, typical for eastern Europe (Fig. 1).

Poznan (52°28'02"N; 16°55'27"E) is a big urban center, covering 261.9 km², with nearly 546,000 residents. The city includes 40.8 km² of forests, 6.5 km² of meadows and pastures and 5.5 km² of parks and green areas. The town is situated in the center of typical farmland with monoculture fields, mainly cereals and oilseed rape. The sampler was situated in the city center, close to a park.

Lublin (51°14'37"N; 22°32'25"E) is an urban center of 147.5 km² and a population of about 342,000 citizens. According to the data of the Central Statistical Office of Poland (GUS, 2015), green areas occupy 16% (24.4 km²) of the city, including 11% forests, meadows and fields (16.8 km²) as well as 6% parks, lawns and green areas around buildings (7.6 km²). The latter number also includes personal private gardens and allotments. Urban area, including buildings and infrastructure,

occupies 84% (128 km²). The aerobiological study was carried out in the vicinity of a park.

Rzeszow (50°02'24"N; 22°00'00"E) covers an area of 116.3 km² and has a population of about 187,000 persons. Green areas occupy 67.1 km², whereas the overall area of buildings and infrastructure equals 49.2 km² (Urban Atlas, 2016). The city is surrounded by a mosaic of forests and small fields, mainly oilseed rape and wheat. The study was carried out in the city center, close to urban green areas.

1.2. Spore sampling and spore counts

The volumetric method was applied in all research points, using Hirst-type samplers (Lanzoni VPPS 2000) located 12–18 m above ground level. Aerobiological samplers operated continuously by sucking 0.01 m³ (10 L) of air per minute, which corresponds to the volume of air inhaled by an average human. The sampler contained a drum rotating at a speed of 2 mm per hour, with one full rotation per week. The drum was covered with Melinex tape coated with silicone or Vaseline dissolved in toluene. The tape was changed once a week at the same hour, and then divided into seven daily segments. Permanent microscopic slides were prepared using glycerol-gelatin stained with fuchsin. Spores were counted using a light microscope (×400), along one horizontal line, 48 mm long. The resulting number of spores was multiplied by a correction factor, to re-calculate the counted number of spores per the whole microscope slide. The results were expressed as average daily concentration of spores in 1 m³ of air (s/m³). The conidia considered in this study were of size range 4–25 × 3–7 μm, mostly oval in shape and with ornamentation typical for *Cladosporium* (Marcinkowska, 2003).

A description of *Cladosporium* spore patterns was compiled for each site including the number of *Cladosporium* spores per month, Seasonal Spore Index (SSI - the total spore count during the study period) as well as the maximum daily concentration and the date of its occurrence. The health hazard to humans was assessed based on threshold values proposed by Rantio-Lehtimäki et al. (1991), D'Amato and Spiekma (1995), and Rapiejko et al. (2004). To illustrate the dynamics of a spore season, we plotted curves of the cumulative sums of the spores. The last point on each curve indicates the total sum of conidia captured in that season.

1.3. Statistical methods

Statistically significant differences between two independent counts of days for which a given spore threshold was exceeded were calculated using the Z-test developed by Zar (1999). The distribution of data and the homogeneity of variance were tested using Shapiro–Wilk (Conover, 1999) and Brown–Forsythe tests (Brown and Forsythe, 1974) respectively. As the distributions were normal and the variances were homogeneous, a parametric ANOVA test was used to detect the differences among the average number of days exceeding threshold values associated with allergy symptoms. The detailed comparisons were made using an LSD (Least Significant Difference) post-hoc test. The chi-squared test was applied to determine statistically significant differences in monthly sums of spores among the sites in each year of study. The statistical hypotheses were tested

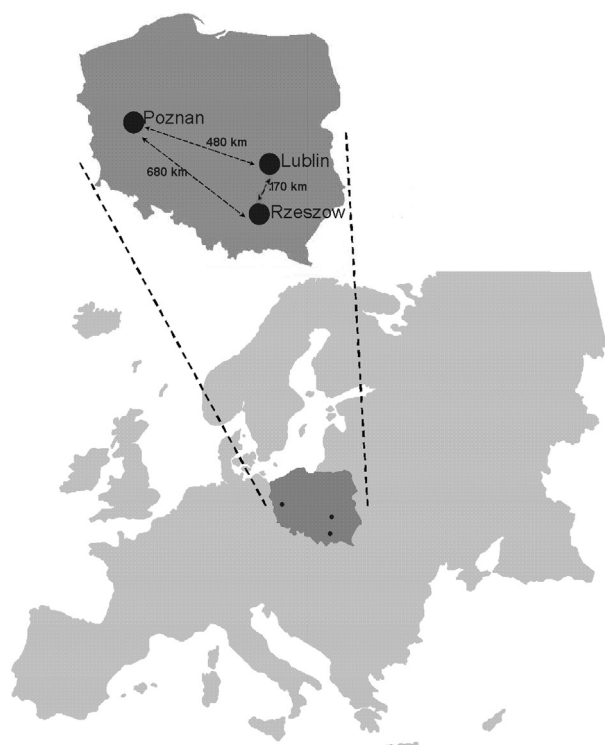


Fig. 1 – The location of experiment sites in Poland, central Europe and distances between the monitoring sites.

at $\alpha \leq 0.05$. Statistical analysis was done using STATISTICA ver. 8 and GraphPadPrism 5.

2. Results and discussion

2.1. Spores and allergens of *Cladosporium* spp.

The biotic components of aerosols are often well adapted to their aerial environment. Pollen grains and fungal spores are moved over long distances, carried by air currents. The spores of *Cladosporium* can migrate in the air not only individually or in colonies, but also as aggregates with particles of soil, dust or other air impurities of an anthropogenic nature (Fig. 2a). Biotic particles occurring in the air can be compared to rafts that are the carriers facilitating the transport of allergens (Nilsson, 1990; Lippmann et al., 2003). Photographs taken during these experiments showed small abiotic particles of unknown composition crystallized on the surface of the conidia produced by *Cladosporium* spp. (Fig. 2b). These particles may damage the structure of spore walls, facilitating entry of chemical contaminants into the protoplasts; the impact of these pollutants may lead to alterations in the properties of allergenic proteins (Ruffin et al., 1983; Nilsson, 1990). In turn, the mechanical damage of spores may lead to an increase in the amounts of allergens released (Ghiani et al., 2012).

Airborne pollutants in gaseous form and particles of dust can alter the concentration, structure and properties of bioaerosol particles. There have been reports concerning the adverse synergistic effect of aerobiological and chemical pollution on human health (Leonard and Lanier, 2008; Sousa et al., 2008). Moreover, it was shown that the surface of airborne sporomorphs can accumulate heavy metals, which then migrate with them, which aggravates the negative effects of these components of aerosols on human health (Duque et al., 2013). It is noteworthy that each successive threshold value published for *Cladosporium* was more stringent. One may speculate that this was partially connected with the increasing chemical pollution of the spores.

2.2. Main characteristics of spore seasons

The seasonal spore index (SSI) of *Cladosporium* spores during the growing season and their maximal values differed significantly (Fig. 3). The most stable results for the consecutive years were obtained in Lublin, especially with respect to the peak concentrations of spores. The highest variation was found in Poznan, where the maxima for SSI and peak numbers of spores in 2011 and 2012 were in opposite years, unlike in Rzeszow, where both the value of SSI as well as the daily maximum was increasing from year to year. The highest SSI in Lublin and Rzeszow were noted in 2012, but in Poznan this index was the highest in 2010. The maximal daily spore count in Poznan was also found in 2010. In Poznan and Rzeszow the maximal concentrations were observed in years having the highest SSI, but in Lublin, the highest peak concentrations of *Cladosporium* spores in the air were nearly the same, regardless of SSI. The value of SSI was always the lowest in Rzeszow. Each year the highest daily peak concentration was found in Poznan and the lowest in Rzeszow. The highest daily peak concentration of *Cladosporium* was found in Poznan in August 2010, reaching 83,102 s/m³. In contrast, the lowest daily peak of spores in Rzeszow was only 13,670 s/m³ and, strangely, it was observed very early in the spore season (May 2010).

2.3. Seasonal sums of spores (SSI)

The average value of SSI obtained in our studies (2010–2012) for the three Polish cities was over 872,000 s/m³. In Rzeszow, Lublin and Poznan the values of this index were 546,000, 990,000 and 1,100,000 s/m³, respectively. A similar yearly sum of spores (865,250 s/m³) was also observed in Lublin in 2002 by Konopinska (2004). The previous report (1995–1996) for Poznan by Stepalska et al. (1999) was only 533,600 s/m³, which is nearly half the amount of spores observed in this study, performed 14 years later. In other Polish cities the reported indices for this taxon in 2004–2013 were much lower; e.g., in Szczecin the average over 10 years was nearly 460,000 s/m³/year, and in Krakow it was over 290,000 s/m³/year (Grinn-Gofron et al., 2016). In studies done in

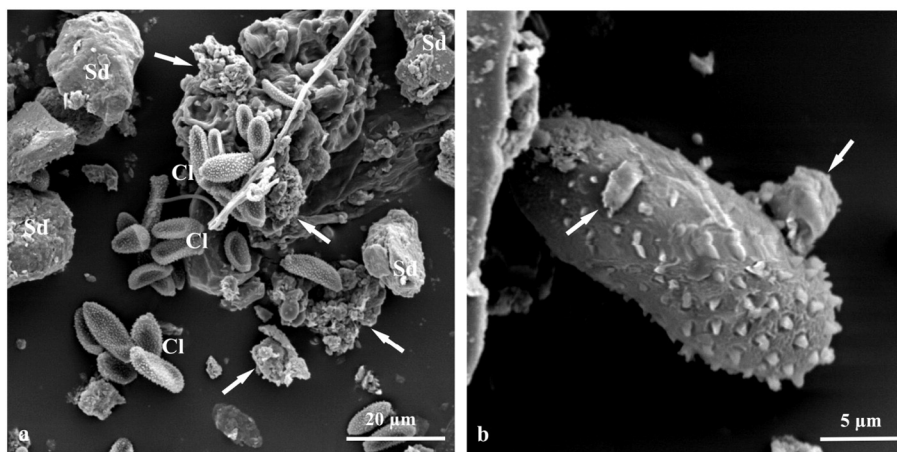


Fig. 2 – Scanning Electron Microscope pictures of conidia of *Cladosporium* spp.: (a) numerous conidia (Cl) surrounded by mineral parts of soil dust (Sd), arrows show condensed aggregates of aerosol of anthropogenic origin; (b) single conidium with visible ornamentation of the cell wall, arrows show the fragments of contaminating particles.

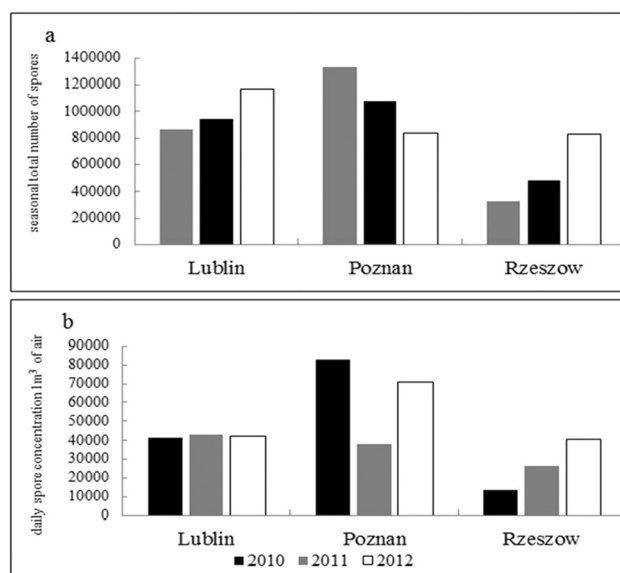


Fig. 3 – Characteristics of the *Cladosporium* spore seasons in three cities in Poland in the period 2010–2012: (a) seasonal total number of spores — SSI; (b) maximum daily spore concentration per cubic meter in each year.

11 cities of Europe by D'Amato and Spieksma (1995) the average annual amount of *Cladosporium* spores was $600,000 \text{ s/m}^3$.

Average annual totals over 2 years of research (2006–2007) for Worcester (UK) were over $676,000 \text{ s/m}^3$ (Sadyś et al., 2016). At 12 monitoring points located in Spain and Portugal, the annual total of *Cladosporium* spp. spores in the majority of the cities ranged from $100,000$ to $200,000 \text{ s/m}^3$, and even up to $300,000 \text{ s/m}^3$ (Rodríguez-Rajo et al., 2005; Aira et al., 2012). An exceptional number of spores ($900,000 \text{ s/m}^3/\text{year}$) was reported in Sevilla, Spain. Lower values of the total annual number of *Cladosporium* spores, as compared to monitoring points in Poland ($220,000$ – $300,000 \text{ s/m}^3$), were also found in Stockholm (Hjelmroos, 1993). The lowest annual counts for these spores were recorded in Norway, where the average sum over six years was nearly $19,000 \text{ s/m}^3$ in Oslo, and this number decreased even more in cities located northwards (Ramfjord, 1991).

2.4. Days exceeding the thresholds of *Cladosporium* spores in the air

A comparison of data obtained for different European countries shows that in Poland the annual sums of *Cladosporium* spores in the atmosphere are among the highest in Europe. In the current study, the highest annual sums of spores and the highest number of days with a concentration exceeding the threshold of 2800 s/m^3 were recorded in Poznan and Lublin, cities located both in western and in eastern Poland. In these cities, over the period 2010–2012, the average number of days in a year exceeding this threshold were 86 and 83 days, respectively. An identical observation (86 days) was obtained for Poznan in 2014 (Grinn-Gofron et al., 2014). In Rzeszow only half of this annual sum of *Cladosporium* spores was found, and there were many fewer days (61) with spore concentrations exceeding the allergy threshold. Previous studies conducted

in 2000–2002 in Rzeszow by Kasprzyk (2008) also showed similar numbers of days with a risk of allergy (56 days on average). Research carried out by Grinn-Gofron (2014) for a period of 10 years (2004–2013) in Szczecin (north-east Poland) showed annual sums of *Cladosporium* spores similar to those in Rzeszow (Szczecin $460,000 \text{ s/m}^3$, Rzeszow $546,000 \text{ s/m}^3$). The number of days posing risk of allergy in these cities was 61 and 51 days, respectively. In 2008 Grinn-Gofron et al. (2008) studied the risk of allergy due to the occurrence of *Cladosporium* spores in the air in 9 Polish cities (this study did not include Poznan, Lublin and Rzeszow). The average number of days per year exceeding a threshold of 2800 s/m^3 was 33; the highest total of days presenting a risk (56 days) was found in Wroclaw (southwest Poland). The risk connected with *Cladosporium* in Warsaw has been reported twice (Grinn-Gofron et al., 2008, 2014) and found to be 44 and 34 days in 2008 and 2014, respectively.

The highest concentrations of *Cladosporium* spores were associated with intensive farming activities in the surrounding regions, which provide plant residues suitable for the development of these fungi. Poznan is located on a big agricultural plain, which could contribute to the highest concentration of aeroallergens. Similar results were previously found for another highly allergenic fungal genus *Alternaria* spp. (Kasprzyk et al., 2013; Kasprzyk et al., 2015; Skjøth et al., 2016).

2.5. Dynamics of spore occurrence in the air

Cumulative curves describing the course of spore seasons showed that in 2010 in Lublin and Poznan, there was a dramatic increase in concentrations from mid-July to mid-August, but thereafter the rate of increase in spore concentrations was lower (Fig. 4). In 2011 and 2012 in April and May the concentrations remained at a low level, but in June a marked increase in spore concentrations was recorded. Different shapes of cumulative curves in these two years were caused by fluctuations in spore concentrations and several peaks that were not observed in 2010. The rapid changes of the curves were caused by short-term (1–2 days) increases in spore concentrations. A clear increase in airborne *Cladosporium* concentrations was observed from the beginning of June in both years. The second period of substantial increase in the spore number was recorded in September. This pattern was the clearest in the cumulative curve for Lublin 2012. The cumulative curves for Rzeszow indicate a gradual increase in spore concentration year by year, evenly distributed across the whole growing season each year. The earliest start to increases in *Cladosporium* spore concentration was seen in 2012, i.e., from mid-April. The curves are not smooth, due to numerous short-term increases and decreases in spore concentrations. In 2010 and 2011 the concentrations increased gradually from the end of May, but the cumulative sum of the spores in the air was much lower.

The greatest sums of *Cladosporium* spores were recorded in the summer months (June–August) and the lowest in April (Fig. 5), but detailed analysis showed that the distribution of the number of spores in each month varied from year to year. Statistically significant differences were found for all monitoring sites (χ^2 test for each location; $p < 0.001$). In Lublin, the

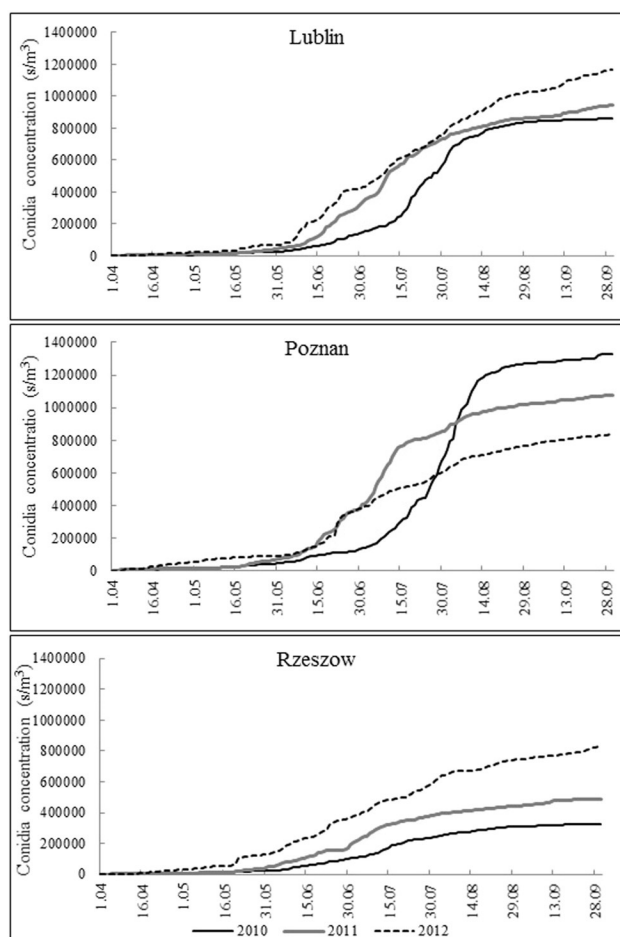


Fig. 4 – The cumulative concentration of conidia of *Cladosporium* spp. in air in three cities of Poland Lublin (central-east of Poland), Poznan (central west), and Rzeszow (southeast), in 2010–2012.

month with the highest spore counts each year was July, i.e., 454,500, 419,000 and 351,000 for 2010–2012. In 2012 a significant number was also recorded in June (351,000 spores). In Poznan the monthly spore totals were also widely different from year to year. In 2010, the largest monthly totals of spores were recorded in July and August, respectively 577,000 and 562,000. In 2011 most spores were found in July (476,000). While in the years 2010 and 2011 the total sums of spores captured in April and September were at similar levels, in 2012 they were significantly higher. In the summer months of 2012, especially in July, the monthly totals were clearly lower compared to previous years; the highest monthly sum of spores was recorded for June, reaching nearly 290,000. In Rzeszow in 2010 and 2011, most spores occurred in July, i.e., 138,000 and 214,000. In the following year, most spores were registered in June (235,000) and a little less in July (232,000). In general, in 2012 the sums of spores in each month were clearly higher than in 2010 and 2011.

2.6. Health hazard caused by the conidia of *Cladosporium*

Different threshold values established for allergic symptoms for the same biological particle may arise due to the differences between individuals from the same population, as well as by

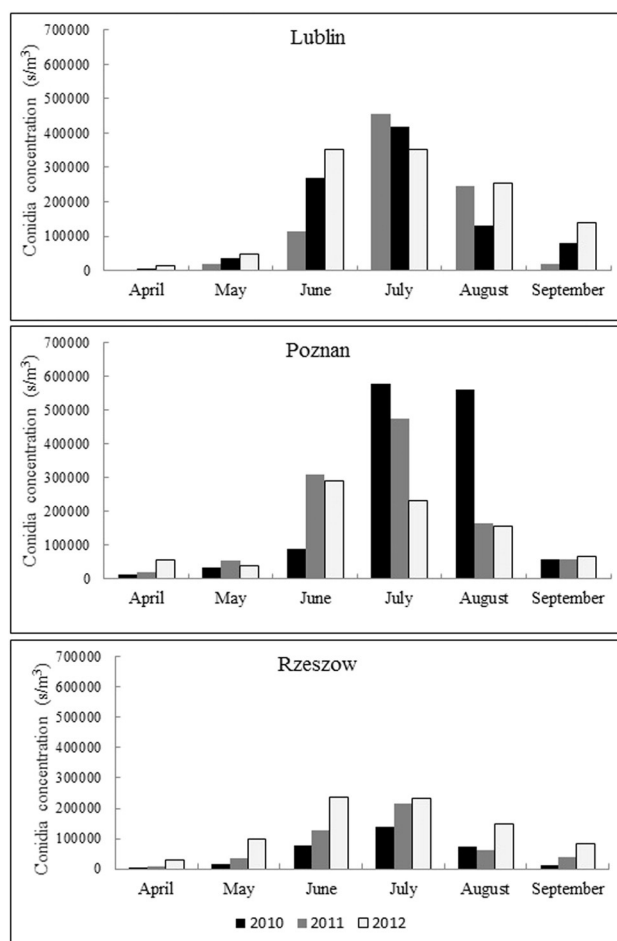


Fig. 5 – Monthly sums of conidia of *Cladosporium* spp. in air in three cities of Poland in 2010–2012: Lublin, Poznan and Rzeszow.

the differences between populations, in relation to sensitization rates. Several papers have already been published on this topic in relation to the exposure of humans to pollen grains, e.g., to hazel, alder, grass, mugwort, plantain, nettle (Rapiejko et al., 2007) and ragweed (Sommer et al., 2015).

The numbers of days exceeding the thresholds of *Cladosporium* spore concentration in air samples were the lowest for the highest threshold value (4000 s/m³), published in 1991 by Rantio-Lehtimäki et al. (Table 1). In the case of the most stringent threshold established for allergic responses to *Cladosporium* spores (Rapiejko et al., 2004), which was set at 2800 spores per cubic meter of air, the number of days in excess of this limit varied from 38 (Rzeszow, southeast Poland, 2010) to 104 (Lublin, central eastern Poland, 2012). In Rzeszow in 2010 and 2011 the number of days above this threshold was significantly lower than in Lublin and Poznan (Table 1). Mean numbers of days exceeding this threshold in the years 2010–2012 ranged from 62 for Rzeszow to 86 days for Lublin. In each city, the highest number of days with spore concentrations exceeding this threshold was noted in 2012 and the lowest in 2010. Similar results were also obtained for the threshold of 3000 spores of *Cladosporium* per cubic meter of air, as reported by D'Amato and Spiekma (1995). Once more the same sites and years constituted the extremes, however the numbers of days were slightly

Table 1 – Number of days exceeding the threshold of *Cladosporium* spp. spores per cubic meter in air samples coinciding with respiratory problems in sensitive patients, according to three research teams.

Year	Lublin	Poznan	Rzeszow	Mean
≥ 2800 s/m ³ (Rapiejko et al., 2004)				
2010	70 ^{ax}	75 ^{ax}	38 ^{bx}	61 ^x
2011	83 ^{ay}	88 ^{ay}	58 ^{by}	76 ^y
2012	104 ^{az}	87 ^{az}	89 ^{az}	93 ^z
Mean	86 ^a	83 ^a	62 ^b	77
≥ 3000 s/m ³ (D'Amato and Spieksma, 1995)				
2010	68 ^{ax}	69 ^{ax}	34 ^{bx}	57 ^x
2011	82 ^{ay}	83 ^{ay}	52 ^{by}	72 ^y
2012	101 ^{az}	82 ^{ay}	84 ^{az}	89 ^z
Mean	84 ^a	78 ^a	57 ^b	73
≥ 4000 s/m ³ (Rantio-Lehtimäki, 1991)				
2010	56 ^{ax}	61 ^{ax}	23 ^{bx}	47 ^x
2011	73 ^{ay}	69 ^{ay}	39 ^{by}	60 ^y
2012	55 ^{ax}	62 ^{ax}	70 ^{az}	62 ^y
Mean	61 ^a	64 ^a	44 ^b	56

Different letters indicate significant differences in the proportion of days in season with concentrations exceeding each threshold value; a, b, c letters refer to the differences between sites (Lublin, Poznan, Rzeszow) in each year; x, y, z letters refer to the differences between years (2010, 2011, 2012) at each site.

Table 2 – Number of days exceeding the thresholds of *Cladosporium* spp. spores per cubic meter in air samples coinciding with respiratory problems in sensitive patients, according to recommended clinical interventions by Rapiejko et al. (2004).

Year	Allergic thresholds spores/m ³	Number of days			
		Lublin	Poznan	Rzeszow	Mean
2010	≥ 2800 –4999 ^a	22	18	22	21
	5000–9999 ^b	21	18	13	17
	10,000–14,999 ^c	9	12	3	8
	≥ 15000 ^d	18	27	0	15
	Daily concentration ≥ 2800 spores ^e	70	75	38	61
2011	≥ 2800 –4999	24	27	25	25
	5000–9999	31	30	26	29
	10,000–14,999	13	8	5	9
	$\geq 15,000$	15	23	2	13
	Daily concentration ≥ 2800 spores	83	88	58	76
2012	≥ 2800 –4999	26	35	34	32
	5000–9999	35	34	33	34
	10,000–14,999	20	11	14	15
	$\geq 15,000$	23	7	8	12
	Daily concentration ≥ 2800 spores	104	87	89	93

Respiratory problems according to Rapiejko et al. (2004):

^a Strong respiratory problems for sensitive patients (requirement for intensive medication).

^b Respiratory problems for patients very sensitive to allergy (drugs required by sensitive patients).

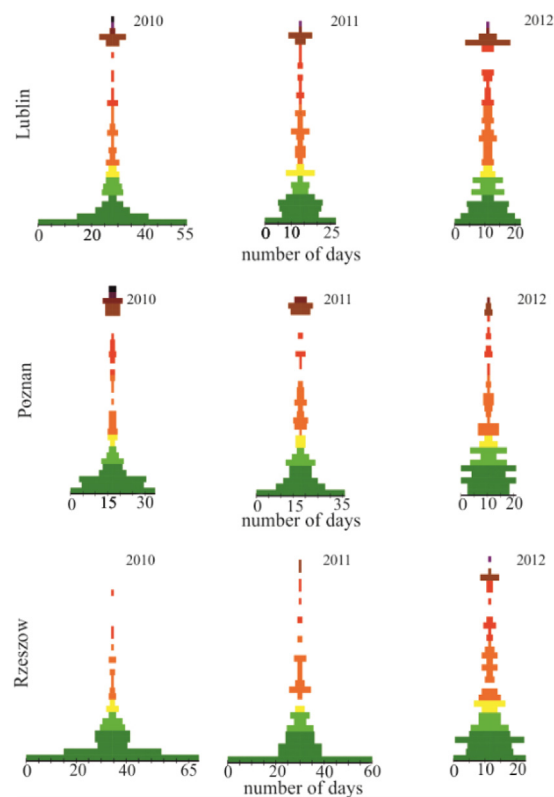
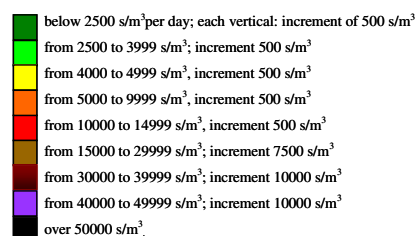
^c Respiratory problems for all patients with allergy (drugs required by all patients).

^d Very strong asthma, difficulties in breathing (hospital treatment required).

^e Threshold for the first occurrence of respiratory problems.

lower: 34 days in 2010 and 57 on average for Rzeszow and 101 days in 2012 and 84 on average for Lublin. Poznan – the city located in the central western part of the country, was intermediate when average numbers of days were considered; however, in two out of three years (2010 and 2011) the number of days exceeding thresholds, as recommended by these two research teams, were the highest. Statistical analyses revealed that in Rzeszow the number of days with concentrations above 3000 spores per cubic meter were significantly lower than in Lublin and Poznan. The threshold of 4000 spores of *Cladosporium* per cubic meter, proposed by Rantio-Lehtimäki et al. (1991), flagged as posing a risk on average 27% fewer days exceeding this threshold, i.e., 56 days, as compared to 77 days calculated according to the threshold established by Rapiejko et al. (2004). The highest average number of days exceeding this threshold was in Poznan (central western Poland), and not in Lublin, as observed for the two lower thresholds. Moreover, in 2012 the highest number of days exceeding this threshold was noted in Rzeszow, which was regarded as the safest place for sensitive

Color codes:

**Fig. 6 – Histogram of daily *Cladosporium* spp. spore concentrations in air at three locations (Lublin, Poznan, Rzeszow) over three years (2010–2012).**

patients, based on thresholds established by Rapiejko et al. (2004) and D'Amato and Spieksma (1995). Summarizing, in 2012 in Rzeszow and Poznan there were many days with very high concentrations of *Cladosporium* conidia, whereas in Lublin, there were many days with numerous spores in the air, but only a few days of extremely high spore concentrations.

2.7. Risk of allergy

The research team of Rapiejko et al. (2004) proposed four spore concentration thresholds; the lowest one allows determination of the number of days dangerous only to patients who are very sensitive to allergy, whereas the next three thresholds were regarded as important for all sensitive patients, leading to moderate, strong or very strong respiratory problems. In

case of the concentration of spores exceeding 15,000 per cubic meter, sensitive patients needed hospital treatment, as they showed very strong symptoms of asthma and difficulties in breathing. Only one site (Rzeszow, 2010) had a year with no days exceeding this highest threshold (Table 2). In the other site/year combinations, the number of days when this threshold was exceeded ranged from 2 to 27 (Poznan, 2010). The highest number of days per year exceeding this upper threshold was twice found in Poznan (2010 and 2011, 27 and 23 days, respectively) and once in Lublin (2012, 23 days). Days with more than 15,000 spores were very rare in Rzeszow and they did not exceed 8 in a given year (Table 2). One-way ANOVA and LSD tests revealed statistically significant differences in the average number of days when the spore concentrations exceeded the highest threshold value in the

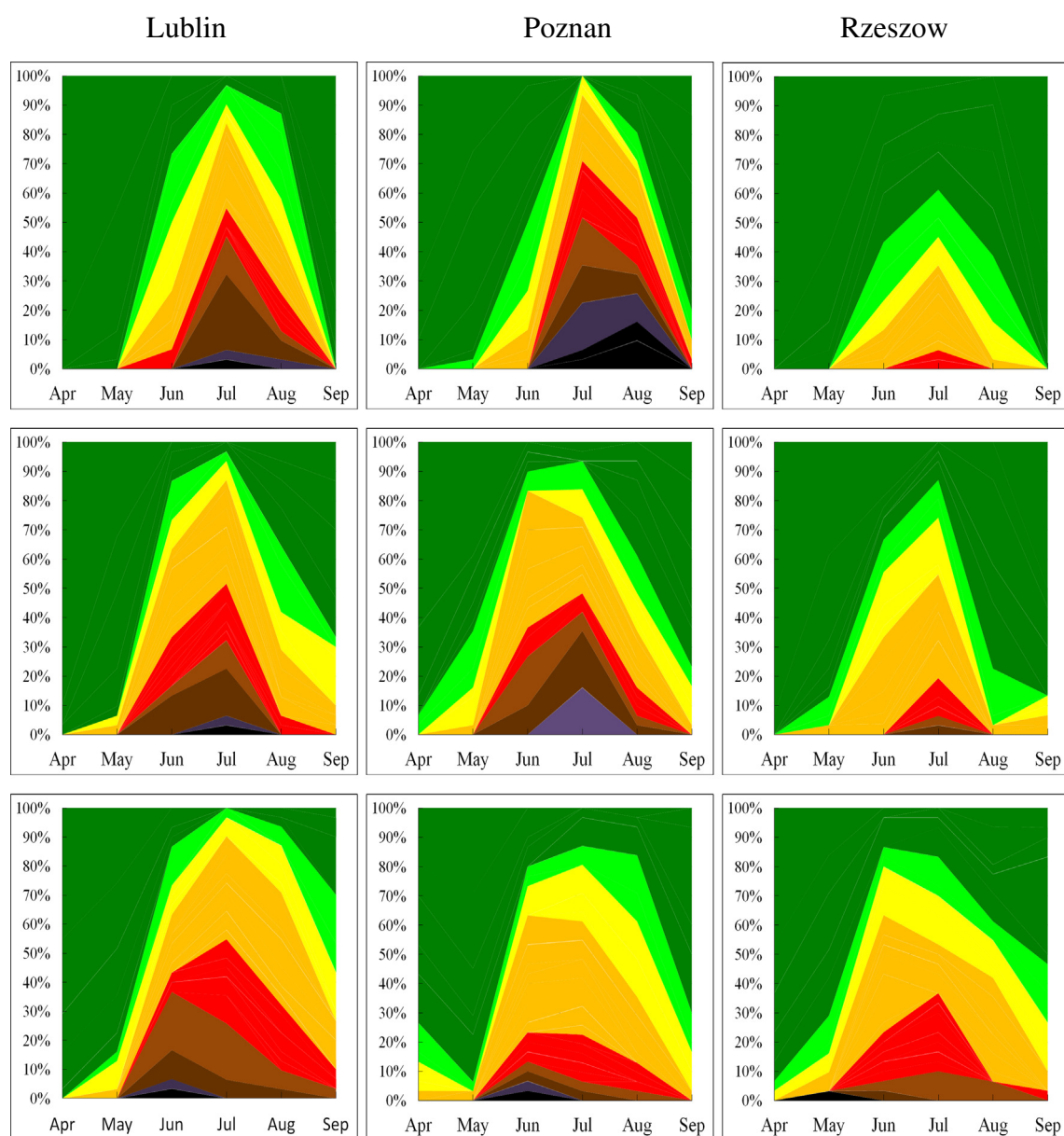


Fig. 7 – Allergy risk related to *Cladosporium* spores in sensitive patients. Three years of study (2010 — upper row, 2011 — middle row, 2012 — bottom row) at three experiment sites in Poland — mean percent of days in the month, from April to September, with *Cladosporium* spp. concentrations (per m³ of air per day) exceeding established clinical risk thresholds. Color codes as in Fig. 6.

studied locations. The number of days with spore concentrations associated with allergy risk as defined by Rapiejko et al. is visualized in Fig. 6, and as seasonal proportions in Fig. 7. The highest number was detected for Poznan (19 days), whereas the lowest was found in Rzeszow (3 days). In 2010 and 2011 the highest spore concentrations were observed in July, whereas in 2012 the same phenomenon was observed early in the season (June) (Fig. 7). However, on average for the 3 Polish cities over 3 years, the highest risk of asthma and respiratory problems in connection to the spores of *Cladosporium* was reported in July (Fig. 8).

2.8. Inhalant allergy caused by *Cladosporium* spores in Europe

In Europe, little is known of inhalant allergies caused by *Cladosporium*. The studies done in 2005–2007 in Portugal (Oliveira et al., 2009) showed that a limit of 3000 s/m³ was exceeded in two cities, including the coastal Porto (4 days) and inland Amares (13 days). In the period covering 27 years in the UK, a threshold value of 4000 s/m³ was exceeded, annually, in up to 55 days in Cardiff and from 30 to 80 days in Derby (Hollins et al., 2004). Monitoring conducted in Worcester (UK) in 2006–2007 showed high risk of allergy (3000 s/m³) in 82 and 47 days (Sadyś et al., 2015). In 1990–1991 in Ankara (Turkey), where the cumulative number of spores per 1 m³ volume of air exceeded 500,000 s/m³/year (Sakiyan and Inceoglu, 2003), there were 54 days above the threshold sensitivity of 3000 s/m³.

The first symptoms of allergy disorders caused by *Cladosporium* are nasal obstructions, but there were no reports on conjunctivitis (Rapiejko et al., 2003). Breitenbach and Simon-Nobbe (2002) warned that the risk of allergy caused by fungal spores exceeds that caused by pollen grains and foodstuffs. The risk of asthma-related deaths was more than doubled when there was a high incidence of ‘mold spores’ (Targonski et al., 1995). Allergenic spores prolonged the suffering of people with inhalant allergies caused by pollen grains and exacerbated the symptoms of allergy (Kasprzyk, 2008). Therefore, there is a high demand for constant monitoring and efficient distribution of information regarding current concentrations of *Cladosporium* spp. This may lead to

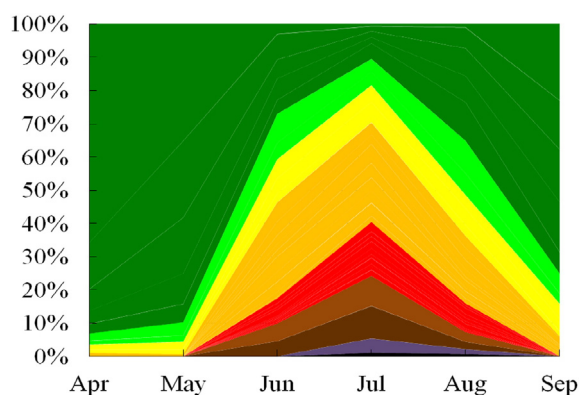


Fig. 8 – Mean percent of days in consecutive months, from April to September, with given concentrations of *Cladosporium* spp. spores in three Polish cities (combined for Lublin, Poznan and Rzeszow). Color codes as in Fig. 6.

the development of statistical models predicting the risk caused by exposure to the spores of *Cladosporium*. However, one must bear in mind that not all advanced models commonly applied in aerobiology can accurately predict the exposure of people to spore-related allergies; this is due to local microclimates, air inversions and strong local sources of spores (Jedryczka et al., 2015).

3. Conclusions

The spores of *Cladosporium* often prevail in atmospheric bioaerosols and occur in high numbers throughout the whole studied period from May to September. The concentrations of spores are often associated with weather and the intensity of agricultural activity, as disturbance of dead plant litter is the primary means by which spores enter the bioaerosol. *Cladosporium* spores are known to contain a high number of different allergenic proteins and will therefore cause allergies in a wide range of people. The policy-making on air quality, for example as proposed by Zhu et al. (2015), should include biological air pollutants, such as *Cladosporium* spp. Knowledge of the actual and forecasted concentration of fungal spores makes it easier to manage the allergy by planning of activities with low exposure to aeroallergens. Information about the concentration of aeroallergens allows for the proper diagnostics, followed by analysis of the effectiveness of medical treatment.

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