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Original Research

On the Microbiological Quality of the Outdoor Air in Poznań, Poland

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Abstract

The microbial quality of the outdoor air in Poznań (Poland) was estimated by sedimentation method in the years 2002 and 2003. Microorganism concentration was high, the average monthly number of bacteria in 2002 often exceeded 3,000 cfu/m^3 and reached in the summer 13,000 cfu/m^3. At least 50% of airborne microorganisms were filamentous fungi. The concentration of two allergenic fungus genera *Cladosporium* and *Alternaria* estimated by Burkard trap reached in summer months 11,000 spores/m^3 in 2002 and 16,000 spores/m^3 in 2003. Airborne microorganism concentrations varied and changed with seasons and years, depending on sampling site and method of determination.

Keywords: outdoor air, microbiological quality, Poznań, *Cladosporium, Alternaria*

Introduction

Microorganisms are present almost everywhere and this is a natural phenomenon. The problem starts when their concentration is high and they find proper conditions for growth, such as high humidity and suitable temperature. Microorganisms present in the air originate from soil, plants and water and atmospheric air is not a convenient environment for the their growth. However, spore-forming bacteria and fungi are able to survive in bioaerosols and stay viable for a long time in the air [1]. Study of airborne microorganisms and their impact on human, animal and plant life is a main interest of a new area of biology and interdisciplinary science - aerobiology.

Many microorganisms present in the air, including viruses, bacteria, fungi, yeasts and protozoa, are associated with diseases occurring in humans, plants and animals. Up to 70% of plant diseases are caused by fungi and there are many microorganisms able to infect animals [1]. It is generally known that microorganisms present in the air can affect human health, causing mainly respiratory and related diseases transmitted via respiratory route. Many species of bacteria as *Streptococcus pyogenes, Mycobacterium tuberculosis* or *Legionella pneumophila* or viruses may cause severe human infections and diseases. However, particularly significant and simultaneously burdensome for humans are filamentous fungi – moulds occupying sometimes 70% of all microorganisms present in the air [2, 3]. They can cause many health problems, including allergic and toxic reactions. Nowadays, allergic respiratory diseases have become common. The number of allergic respiratory diseases is increasing and therefore allergies are called " the epidemic of the 21st century" [3]. Now atopic allergy affects over 25% of the population in the industrialized world and it is estimated that in the year

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2020, 50% of total world population will be affected by different allergies [4]. The basic reason for this dramatic rise of allergic diseases is urban air pollution and therefore it is observed first of all in the industrialized world [2-5]. The major allergic diseases induced by fungi are asthma, rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonia [4, 6]. The allergen exposure may be via inhalation, skin contact, enteral or parenteral introduction. It has to be stressed that dead moulds are as toxic as live ones as they can still make people sick. Development of an allergic disease is conditioned by interrelation between individual genetic factors of a person and environmental agents. Particularly sensitive to allergens are the elderly, infants, people on chemotherapy, AIDS patients, etc. Many genera of fungi can cause allergic responses, but particularly important are some species of *Cladosporium, Alternaria, Penicillium,* and *Aspergillus*. *Cladosporium* and *Alternaria* are frequent and predominant genera present mainly in the outdoor air and found throughout the world, whereas *Penicillium* and *Aspergillus* species are generally isolated from indoor environments [7, 8]. Some moulds also produce mycotoxins. Some mould species become particularly toxigenic by producing dangerous volatile compounds [3, 7].

Monitoring microbiological contamination of air has received increasing attention. A research group from the University of Heraklion-Crete in Greece compiled data for thousands of athletes participating in the 2004 Olimpic Games in Athens to ensure safety for allergic persons. Information is based on a 15-year database on aeroallergens (pollen and fungi spores) present in the air of three Olympic cities. The 2004 Olimpics expected a relatively high concentration of *Alternaria* and *Cladosporium* [9].

The concentration of microorganisms in atmospheric air and their qualitative composition is very much dependent on different atmospheric factors [10-13]. Wind, rain, sunlight, ozone – all these natural forces have a dispersing and sometimes even bacteriocidal affect on microorganisms [11]. Also, chemical air pollution is associated with microbiological contamination [5, 12].

Nowadays people spend most of their lives (80-90%) indoors, so indoor air quality is important for human health and comfort. Long-term contact of people with some species of microorganisms can be a source of serious illnesses, can influence a person's mental power and learning ability. Microflora of indoor air is influenced significantly by the occupants, equipment, building materials, dust, air conditioning systems and many other sources present in enclosed spaces. On the other side, in an ideal world, microorganism composition of indoor air would reflect qualitatively that of outdoor air with quantitative difference only. Therefore outdoor air is thought to be an important source of microorganisms indoors and monitoring of outdoor air may become a significant element necessary for the estimation of indoor air quality [13, 14,15].

The aim of our work is to perform a long-term observation of microbiological quality of both outdoor air in the city of Poznań and indoor air in selected rooms located in the center of the city. This paper presents preliminary results of the outdoor air monitoring in the first period of the investigation, including 2002 and 2003 (the whole project embraces 5 years of study: 2002-2006). Poznań, located in west Poland (300 km west from Warsaw and 300 km east from Berlin), has a population of about 600,000.

Materials and Methods

Outdoor air monitoring was performed in Poznań in two sampling sites ca 3 km apart. One point, called Esculap, was situated in a complex of loose buildings, surrounded by parks and gardens and the second sampling site, Altum, was located in the center of the city. In both measuring places air samples were taken at two heights – lower level (1 m above the ground) and upper level (36 m (Esculap) and 80 m (Altum) above the ground).

The monitoring of outdoor air quality was performed in the period from April to October in 2002 and 2003.

Total Number of Bacteria and Fungi

The concentration of bacteria and fungi was determined using the conventional sedimentation method with Petri Dishes. Meteorological conditions in Poznań were as follows:

The prevailing winds from west and southwest, mean wind speed was 3.8 m/s in 2002 and 3.6 m/s in 2003. Mean temperature in Poznań was 9.7°C in 2002 and 8.9°C in 2003, mean relative humidity 79.5% and 75.8% in 2002 and 2003, respectively. Precipitation was 588 mm in 2002 and 408 mm in 2003 and annual number of hours of insolation was 1831 in 2002 and 2005 hours in 2003.

Petri dishes were exposed to the outdoor air for 10 minutes and then incubated for 24-48 hours at 37°C (enumeration of bacteria) and up to 10 days at 25°C (enumeration of fungi).

Sabouraud Agar medium with 2% glucose was used for quantification of fungi, and yeasts extract agar for bacteria. Both media were purchased from Merck in Darmstadt, Germany

Fungal isolates were identified according to Fassatiova key [16].

The Concentration of *Cladosporium* and *Alternaria* Spores

Outdoor spores of *Alternaria* and *Cladosporium* were collected by Burkard 7-day volumetric sampling trap [17]. The Burkard sampler is an air-suction device (10 liters per

minute) capable of gathering continuous samples during the fungal spore production season.

The trap was mounted about 36 m above the ground (72 m above sea level), in the sampling site Esculap. Melinex tap covered with an adhesive mix of glycerin, vaseline, liquid paraffin and toluene was cut into 7 daily lengths and mounted in gelvatol on glass microscope slides. Daily readings were taken for 1 horizontal strap, spore counts were then converted to the number of spores/m^3 air as a daily mean value [18].

Results

Quantitative Measurements of Microbial Air Contamination

The outdoor air in the city of Poznań according to our results taken in 2002 and presented in Fig. 1A is quite contaminated by microorganisms. Polish Standards [19, 20] for outdoor air define air contamination as medium when the number of bacteria in cubic meters is lower than 3,000 and exceeding this limit means the air is highly contaminated. According to these standards the concentration of fungi above 5,000 cfu/m^3 may negatively affect human health. In the investigated period the concentration of bacteria was very often higher than 3,000 cfu/m^3 and concentration of fungi above 5,000 cfu/m^3. In 2002 maximum contents of fungi, bacteria and yeast took place in July and August. And at this moment the concentration of moulds reached the high number of 16,000 cfu/m^3, but also a concentration of bacteria was the highest (13,000 cfu/m^3). And in the period of July-September the quality of outdoor air in Poznań in 2002 could be classified according to Polish Standards as highly contaminated and threatening the human environment [19]. However, the measurements performed in the same place and in the same period (April-October) but one year later (2003) presented numbers far lower than in 2002, i.e. maximal average monthly concentration of moulds was 3,500 cfu/m^3, and bacteria 3,000 cfu/m^3 (Fig. 1B). Likewise, a time profile changed, the highest concentration of moulds was found in July, whereas the highest number of bacteria was observed in August but also in June when the bacterial concentration reached a high value of 2,700 cfu/m^3. The concentration of bacteria was unexpectedly high on April 24th, 2003 but this was a single measurement on the day of extremely favorable weather conditions (+18.7°C, no wind, no rain, sunny).

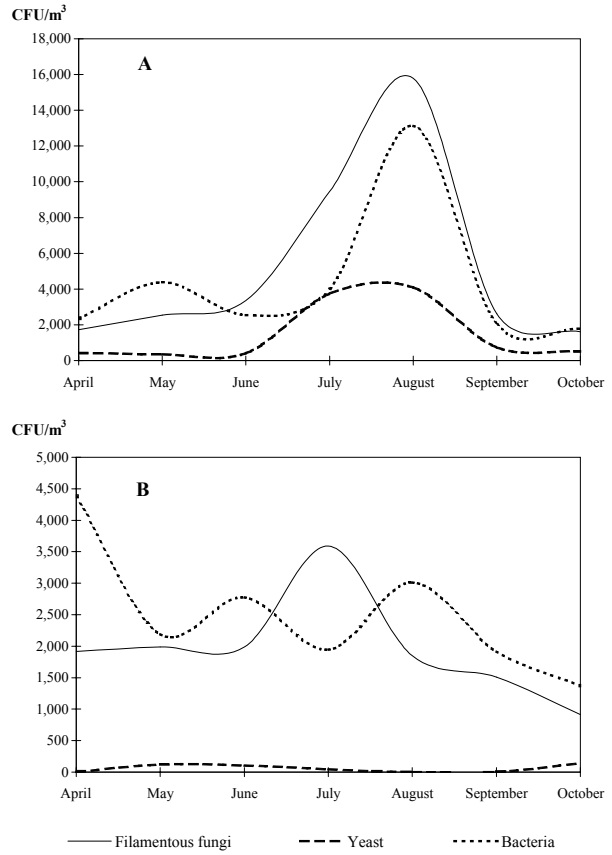

Fig.1. Total number of microorganisms monitored in 2002 (A) and 2003 (B) at sampling site Esculap, at 36 m. Data present the average monthly concentration except data for April, when only one measurement (24.04) was performed.

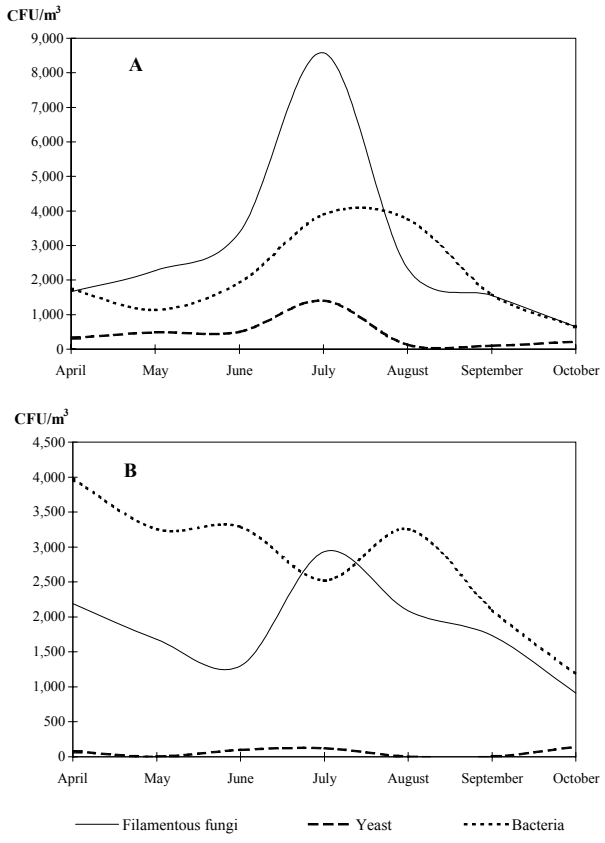

Fig. 2. Total number of microorganisms monitored in 2002 (A) and 2003 (B) at sampling site Altum at 80 m. Data present the average monthly concentration except data for April, when only one measurement (24.04) was performed.

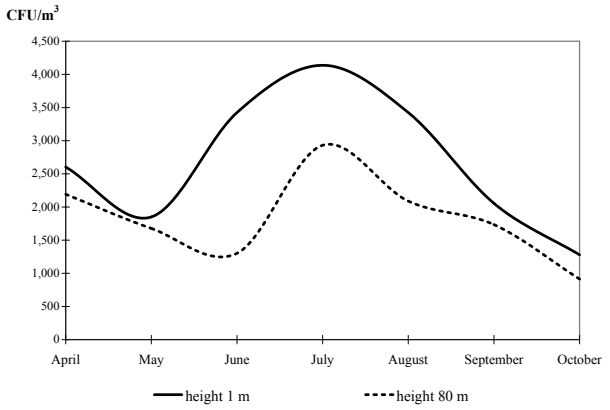

Fig. 3. Total average number of fungi observed in 2003 at a sampling site: Altum at the height: 1 m and 80 m above ground.

Air monitoring also was performed at a second sampling site - Altum, located directly in the centre of the city (Fig. 2). This point registered in 2002 a totally lower level of microorganism concentrations than in the sampling site Esculap. However, in 2003 the difference was indistinguishable (Figs. 1 and 2). The highest concentration of moulds found in Esculap in 2002 reached almost 16,000 cfu/m^3 towards the value of 9,000 cfu/m^3 at point Altum. Bacterial concentration in 2003 was kept rather steady with only two little slopes in the summer.

All data presented in Figures 1 and 2 were taken at the upper level, i.e. at the height of 36 m (Esculap) and 80 m (Altum) above ground. Air samples in both sampling sites were also taken at the lower level, i.e. 1 m above ground. Average monthly values of microorganism concentrations observed at the height of 1 m above ground were on average 40-50% higher than results taken at the upper level and the difference between data taken at two levels was wider when the number of microorganisms was higher (Fig. 3).

Reliable data on microbiological quality of both outdoor and indoor air in Poland is very limited. One of the last reports concerns the concentration of microorganisms in the air of the Gulf of Gdansk, a coastal zone in the southern Baltic [21]. The most abundant microorganisms in this region were fungi, their concentration in the summer (August) reached 1,100 cfu/m^3. In Łódź, a big city in central Poland, the concentration of fungi found varied from 770 to 1,200 cfu/m^3 and predominating genera were *Aspergillus* and *Penicillium* (over 50% of all fungal genera) [22]. According to some world data the fungal spore count in outdoor air is on average 230 spores/m^3 [4]. More information on microbial air contamination all over the world is collected in the vicinity of wastewater plants, where permanent air monitoring is obligatory [23] and in farming environments where workers are exposed to many different microorganisms [24, 25].

The data presented above are average monthly values obtained from 4-5 weekly measurements in each month, except April, when only one assay (24.04) was performed. The scattering of data in the investigated period from May to October was usually very wide, for example the average fungal concentration in July 2003 observed in a measuring point Esculap at the upper level was 3,500 cfu/m^3, whereas on July 15th the concentration of fungi at this point reached 7,000 cfu/m^3. This way of presentation does not reflect namely all precise values of microorganism concentrations registered each day in the investigated period; however, it makes it possible to observe a global tendency, total changes of microorganism concentrations in the air in periods of months and years.

Fungal Composition of Microbial Air Contamination

Moulds participate significantly in microbial air contamination in Poznań. In 2002 the concentration of fungi exceeded bacterial contents across almost all year and in the next year the contribution of fungi and bacteria was on average equal. 38% of the total number of filamentous fungi found in 2002 in the centre of Poznań was occupied by the genera *Cladosporium spp.,* other genera of fungi had a much lower contribution (Fig. 4A), i.e. *Mucor spp.* – 12%, *Alternaria spp.* – 8%. The concentration of the predominating mould (also *Cladosporium spp.*) in 2003 increased even to 57%, the number of *Mucor spp.* was

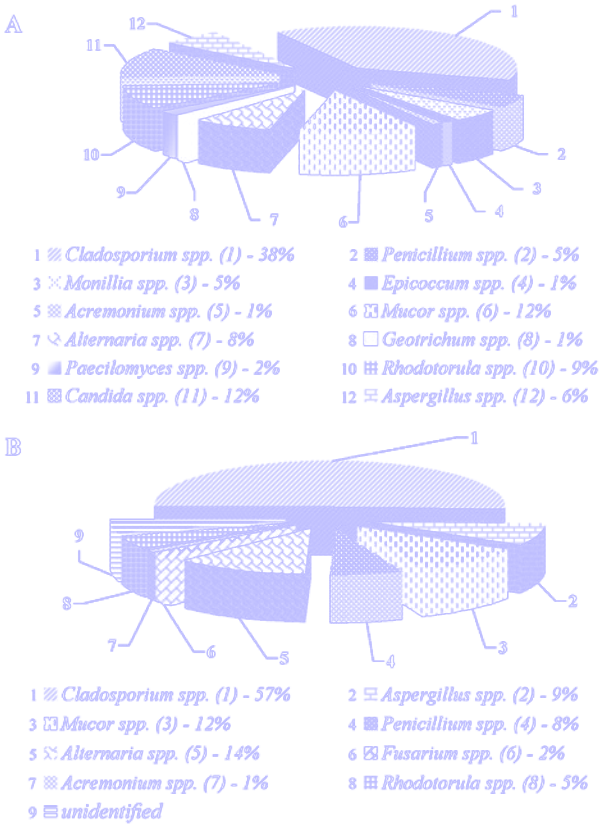

Fig. 4. Fungal composition found in 2002 (A) and 2003 (B) in the outdoor air in the city of Poznan at the sampling site: Altum. The calculation is based on the average monthly values throughout the all investigated period.

exactly the same, and a contribution of *Alternaria spp.* increased to 14% which placed this genera on the second predominating moulds (Fig. 4B).

Presented data confirm the general observation that both *Cladosporium* and *Alternaria* are predominating genera in outdoor air. In the city of Eskisehir in Turkey two species *Alternaria alternata* and *Cladosporium cladosporioides* were the most abundant (13.66% and 5.80 %, respectively) during the measurements performed from March to November 2001 [26]. Further observation in Poznań would answer the question if the contribution of allergenic microorganisms *Cladosporium* and *Alternaria* in the atmospheric air is still significant and increasing like it could be concluded from data taken in 2002 and 2003.

Estimation of *Cladosporium* and *Alternaria* Spore Concentration by Burkard Trap

The concentration of *Cladosporium* and *Alternaria* is a crucial parameter of microbiological quality of air and both fungi are objects of interest all over the world because of their negative influence on human health. *Cladosporium* and *Alternaria* live as saprophytes or as parasites on many kinds of plants. *Cladosporium* is the most common airborne fungus in the temperate zones. *Alternaria* is the predominant fungus demonstrated in warm and humid climate [4]. Both are strongly associated with asthma and other respiratory diseases [10, 11]. In Taiwan, 45% of asthmatic children and 70% of asthmatic adults had positive allergic responses to moulds [12]. In rural towns of New South Wales in Australia approximately 1 in 10 children have asthma and 1 in 7 children are allergic to the fungus *Alternaria* [27]. Also, *Cladosporium* is a source of allergens. In the study performed in Asthma Center in Brooklyn, New York, USA, a strong positive association was found between asthma severity and *Cladosporium* sensitivity for adults but not for children [8].

In this work all results presented above including also quantitative contribution of moulds genera were collected by a sedimentation method with the use of Petri plates exposed to the examined air for 10 minutes. The calculation in this method is based on the assumption that during 5 minutes all microorganisms present in 100 dm^3 of the air are deposited on the Petri plate of the 100 cm^2 surface. It means that obtained results are only half quantitative estimation. However, the sedimentation method is simple, cheap and very popular. More precise results can be obtained using some other methods, including different commercially available sampling devices, like for example Burkard volumetric sampling trap based on Hirst trap described in 1952 [17]. Particularly, the concentration of fungi spores is now monitored mainly by such volumetric traps. Also in this work, concentrations of two fungus genera - *Cladosporium* and *Alternaria* was measured by the Burkard trap installed in the measuring point Esculap and results obtained in the period April-October 2002 and 2003 are presented in Figs. 5 and 6.

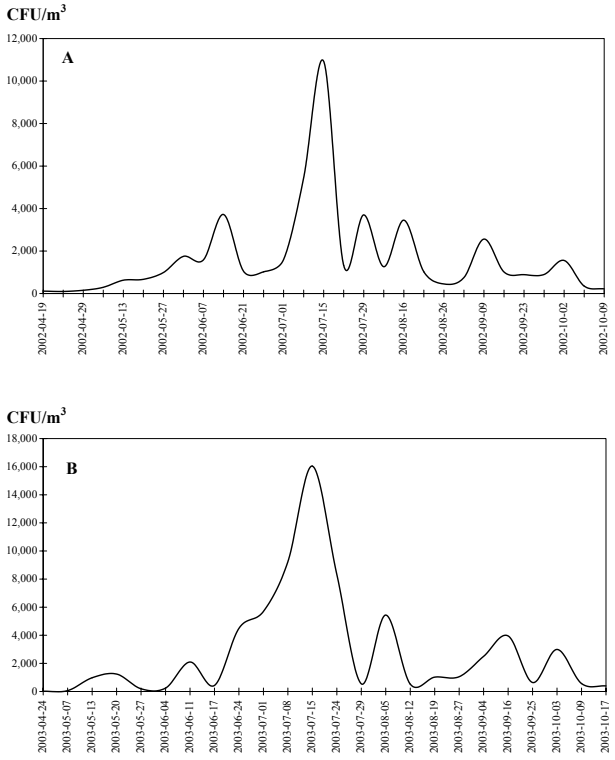

Fig. 5. Concentration of *Cladosporium* spores in the outdoor air in the years 2002 (A) and 2003(B) in the city of Poznań.

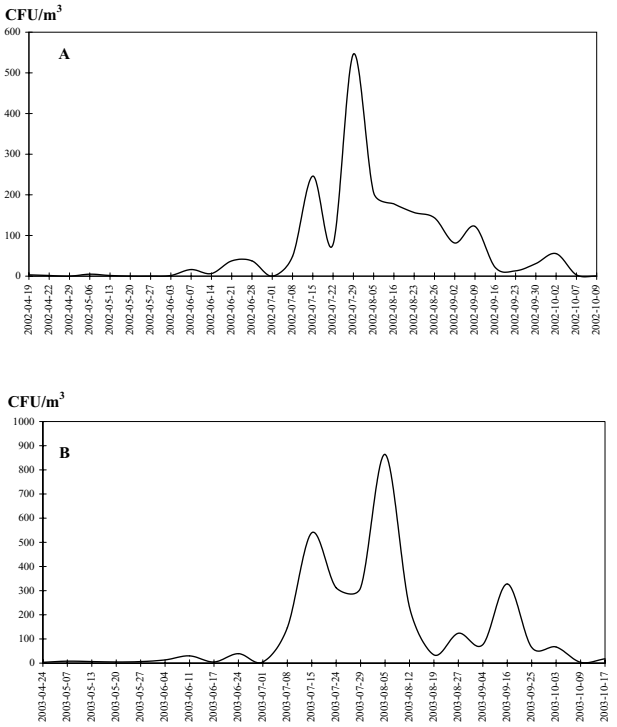

Fig. 6. Concentration of *Alternaria* spores in the outdoor air in the years 2002 (A) and 2003(B) in the city of Poznań.

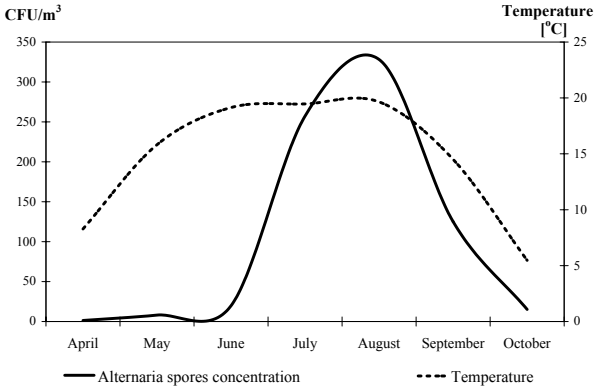

Fig. 7. Concentration of *Alternaria* spores and the average temperature in Poznań in 2003.

It is easy to notice that the concentration of *Cladosporium* and *Alternaria* spores is changing throughout the year and attains a peak in the summer. In 2002 this highest value of *Cladosporium* was 11,000 spores/m^3 and in 2003 the mould concentration reached even 16,000 spores/m^3. In the rest of the observed period in both years *Cladosporium* spores count varied from a few hundred to ca 4,000 per m^3. The demonstrated level of *Alternaria* is far lower, and only in the peak at the beginning of August (05.08.2004) did it reach the number of 864 spores/m^3. Other investigations in the cities of Derby (United Kingdom), Cracow and Poznań in Poland in 1995-2002 reported much higher concentrations of *Alternaria* exceeding even occasionally 6,000 spores per cubic meter of air [7]. The mean daily spores count of *Alternaria* found in a rural town of New South Wales Australia was 300.6 in summer and 30.4 in winter [27].

Annual fungal spore concentration varies considerably between years and is related to weather conditions. Data collected in Great Britain over 27 years indicated that the number of days in summer when the *Cladosporium* spores are above the allergic concentration is positively related with regional temperature and negatively correlated with precipitation [28].

Poznań is located in the region of moderate climate, with temperatures in the summer months often above 20°C and rarely exceeding 30°C. Analysis of weather conditions in Poznań in 2002 and 2003 indicates clearly that an increase of the temperature supports a rise of microorganism concentrations. A typical example of such a relation is shown in Fig. 7, where the average concentration of *Alternaria* in 2003 is compared with an average temperature profile. Similar relationships took place in 2002. The highest concentration of *Alternaria* and *Cladosporium* spores was found at the highest temperatures of the air.

Conclusions

Microbial contamination of the outdoor air in Poznań is relatively high, according to Polish Standards [19, 20] and in comparison to pre-cited other studies [4, 21, 22, 27]. At least 50% of airborne microorganisms are fungi and particularly disquieting is a large contribution of allergic moulds *Cladosporium* and *Alternaria.* The concentration of *Cladosporium* in the atmospheric air of Poznań estimated in one sampling site by Burkard trap in summer 2003 reached 16,000 spores/m^3. However, the concentration of microorganisms in the atmospheric air varies, changing significantly two consecutive years: 2002 and 2003 (results obtained differed depending on sampling sites and measuring method). Microorganism concentrations was also strictly associated with the air temperature with maximum values in two summer months. Microbial quality of air should be continuously monitored for long periods of time.

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