Pol. J. Environ. Stud. Vol. 27, No. 1 (2018), 257-266
DOI: *10.15244/pjoes/75201* ONLINE PUBLICATION DATE: 2017-12-01

Original Research

Aeromycological Monitoring of Disused Mines in Poland

Wojciech Pusz[1], Jakub Grzeszczuk[1]*, Paweł P. Zagożdżon[2], Włodzimierz Kita[1]

[1]Plant Pathology and Mycology Division, Department of Plant Protection, Faculty Of Life Sciences and Technology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland
[2]Faculty of Geoengineering, Mining and Geology, Wrocław University of Science and Technology, Wrocław, Poland

Received: 30 April 2017
Accepted: 21 June 2017

Abstract

The aim of the study was to determine the types of changes – depending on season and tourist traffic – occur in the array of fungi in selected adits in Lower Silesia (Ciechanowice, Rozdroże Izerskie or Izera Crossroads, Kletno – Sztolnia Wiejąca or Windy Adit, and Kletno – Sztolnia Turystyczna or Tourist Adit). Air samples were collected by impact method in 2015-16. The dominant fungi in the adit air were the ones of the genus *Penicillium* and of the species *Cladosporium cladosporiodes*. The mycobiota of each adit varied to a certain extent; the differences, however, were more pronounced between the adits, which are inaccessible to the public and the Tourist Adit in Kletno. It was demonstrated that the presence of tourists exerts a marked influence on species composition and the number of fungi and spores in caves and underground facilities. This may greatly affect the biological and historical maintenance and stability of these sites.

Keywords: airborne fungi, mycobiota, bioaerosol, mycotoxins, speleomycology, Poland, Sudety Mountains

Introduction

Aeromycology is the study of the intensity of aerial dispersal of organic matter such as pollen and fungal and/or bacterial spores, both indoors and outdoors [1] (Carinanos, 2004, Aerobiology as a tool to help in episodes of occupational allergy in work places). One of the main concerns of aeromycological research is to identify fungal spores that may pose a threat to humans [2-3]. The ambient air may additionally contain fungal organs, namely propagules, which are capable of producing mycotoxins that are harmful to people and animals [4-5]. One other aspect is that the findings provide early warning of a plant disease [6]. In recent years, scientists have employed aeromycological methods for studying extreme ecosystems such as caves [7].

Caves, adits, and other man-made underground structures create a hostile environment for living organisms, on account of which such places become paradoxically attractive for many fields of research, including microbiology and mycology [7-8]. Researchers are trying to document the effects of microorganisms on the ecosystem of caves as a whole, and seeking species or strains that may be used in industry [9]. Notwithstanding the considerable technical difficulties such places understandably present, there are good grounds for aeromycological researchers to continue investigating them [8]. The findings may determine whether the concentrations of fungal spores reach a critical level that will adversely affect cave ecosystems and, as a result,

*e-mail: jakub.grzeszczuk@upwr.edu.pl

pose a threat to, e.g., cave formations [7], animals [10-11], or even historical treasures [12]. It is now often acknowledged that there is a greater need for a universal monitoring system for underground sites, in particular those that are frequently visited by tourists: this system seems to be the only one capable of detecting negative changes occurring in underground ecosystems [13]. It can be strongly argued that aeromycology could become instrumental in such monitoring, as it can efficiently deliver results that demonstrate how the fungal make-up in cave air is affected by the presence of tourists. If it were likely to trigger unwelcome changes, the number of visitors could temporarily be limited or the cave closed so that the make-up of the ecosystem could either fully or at least partially be restored [14].

The aim of the study was to document how seasons of the year and tourist traffic trigger changes in the array of fungi in selected adits in Lower Silesia.

Materials and Methods

The samples were collected four times during the year, in spring, summer, autumn, and winter of 2015-16 in four disused mines: Ciechanowice, Rozdroże Izerskie, Kletno – Sztolnia Wiejąca, and Kletno – Sztolnia Turystyczna in Lower Silesia (southwestern Poland) (Fig. 1).

Study Area

Lower Silesia, the Sudetes in particular, is geologically an exceedingly complex area. Traditionally it is referred to as a mosaic: a mixture of contiguous geological formations of diverse lithology, age, and evolution [15]. It is rich in mineral resources, which have been exploited since the Middle Ages. As a result, its mountains are dotted with hundreds of old subterranean mine workings that have created interesting ecosystems developing highly fertile mineralogical and chemical substrates and varying in both hydrological conditions and composition of air [16].

The sites described below are mines dating back to the 20th and, most likely, 19th centuries, which remained after intensive extraction of a range of ores. One of the adits is now open to the public as an underground tourist route, the others are the subject of scientific investigation, and they are often visited by potholing enthusiasts.

The Ciechanowice adit, near Jelenia Góra, is part of the unique remains of a mine-producing crystalline limestone used in lime production. It is situated on a steep, 40-degree slope, approximately 50 m above the River Bóbr. The adit is 90 m long, 1.5-1.8 m wide and, on average, 1.8 m high. At the end, there is a cavernous five-m-high chamber, measuring 5x30 m.

The adit was driven through greenstones and greenstone schists, which are part of the Kaczawa Metamorphic Belt. These are metamorphic silicate rocks made up of mainly feldspars and chlorites. The walls of the exploitation chamber are covered predominantly with fair crystalline limestone: a rock composed of calcite or, strictly speaking, calcium carbonate. There are a number of sizeable tectonic faults visible in the adit.

The site is generally dry except at the very end, where there is a permanent seepage of water. Several calcite speleothems can be seen on the walls. There is only one entry point so air currents are practically undetectable [17].

The Rozdroże Izerskie adit started operating in 1960-61 following the location of a quartz vein. It is situated on the slope of Mount Izerskie Garby, where this, as well as other rocks, was intensively quarried from the Stanisław Quartz Mine until 2001. The mine consists of a 200-m-long main adit and seven 10-40-m-long side drifts. The adit is quite sizeable at three m wide and 2.5 m high, which is typical for a 20th century mine.

The adit is located in the Izera Massif [15] and most of it cuts through a quartz vein containing around 98.8% of SiO_2 and through high-concentration silicon dioxide (silica) feldspar-quartz rock. Occasionally other components cane be identified, namely schists and alkaline vein igneous rocks and lamprophyres.

A number of drippings and minor seepage are noticeable in the roof and sidewalls. This has caused the flooding of more than 200 m of the workings, with water rising up to approximately one meter, and a continuous seepage of water at the rate of one litre per second from the entrance of the adit. Airflow is severely hindered because of only one entrance and an intricate network of galleries.

Kletno, in Lower Silesia, has a long and rich history of mining. Iron, silver, copper, and fluorite ores were extensively extracted from as early as the 14th century. The scale of the work was increased after World War II, when extraction of uranium ore was in progress in 1948-53, and of fluorite until 1958. Centuries of mining have left 20 adits at 10 levels and three shafts, whose total length is over 37 km. Some of them have been made accessible for scientific research [18].

The historic mine in Kletno revealed a very interesting geological feature: a highly mineralised fault zone in the metamorphic Orlica-Śnieżnik Massif [15]. The zone, traditionally referred to as the Kletno Thrust Sheet, separates gneiss formations from schists, including graphite, marbles, and skarns, as well as several pockets of quartz and fluorite. It is also unique in terms of mineralogical composition, owing to immense diversity of polymetallic mineralisation. Accordingly, the mineral substrate in the adits is tremendously diversified: it is composed of silicate rocks, including pockets of pure quartz as well as carbonate rocks; a subsequent second mineralisation produced mainly magnetite and also other oxides, selenides, and sulfides: altogether around 60 minerals [19].

Situated in the higher part of a side valley in Kletno, adit number 18, *Sztolnia Turystyczna*, has been open to the general public as "the historic uranium mine in Kletno." The aggregate length of the workings is a mere 300 m, but their layout is exceedingly complex. There are two entrances, a drift mouth and a shaft mouth, which ensure sufficient ventilation of the workings, most of which are two meters wide and 2.5 m high. The adit has been driven mainly through a large pocket of quartz with formations of, among others, fluorite and magnetite lodes. Some galleries run through marble, where karst features are easily noticeable in places, and through gneiss and graphite schist. The rock mass is fractured and crushed as a result of periodic intense tectonic activity. In places, fairly strong seepage of water from the roof occurs.

The mouth of adit number 12, Sztolnia Wiejąca, is situated in the lower part of the valley. The total length of the accessible workings exceeds 300 m: 180 m for the main gallery, the rest for side drifts. Their state of repair and their size vary significantly: the well-preserved sections are 1.5-2 m wide and approximately 2.5 m high, and where there were incidents of cave-in or roof fall the width increases to 3.6 m while the height is reduced to hardly one meter. There is massive air flow resulting from natural ventilation in the main working, increasing during warm spells; this process occurs in the contact zone of gneiss, schist, and erlan-rock.

Fungal Identification and Mycological Evaluation of the Air

The air sampler (Air Ideal 3P) was programmed for air sample volumes of 50, 100, and 150 l. The air samples were collected from one location outside the adit (near the entrance) and three locations inside (mine entrance, twilight zone, and dark zone).

Measurement in every study site was performed in six replicates for each volume. The sampler was positioned 1.5 m above the level of the floor. The incubation of the cultures was carried out at 15ºC and room temperature (22ºC) for 4-14 days in darkness. Potato dextrose agar (PDA, Biocorp) medium was used for the isolation of fungi from the air and for the identification of some species, while Czapek-Dox agar (1.2% agar, Biocorp) and malt extract agar (MEA, Biocorp) were used for identifying *Penicillii* and *Aspergillii*. After incubation, the fungal colonies grown on each one of the Petri dishes of 90 mm in diameter were counted and identified.

Precise identification of the sampled fungi was enabled by macro- and microscopic observations; the focus was turned to hyphae, conidia, and sporangia of the colonies that had grown on culture media. The filamentous fungi were identified by adopting diagnostic keys and descriptions by Pitt and Hocking [20] and Watanabe [21].

The extent of ecological diversification was analyzed by computing such ecological indicators as Margalef's, Simpson's, Shanon-Wiener, and Pielou indices [22-23]. The obtained means were analyzed by ANOVA using Statistica 12.0 package, and they were compared using Tukey HSD (honest significant differences) test at $\alpha \leq 0.05$.

Results and Discussion

The results revealed that the number of airborne fungal spores varied enormously depending on the season, and confirmed conclusively that there is a strong link between species diversity and the time of measurements as well as the features of the site under study. More detailed data are provided in Tables 1-4 and they are interpreted in four sections, each relating to one of the four earmarked sites.

Ciechanowice

The adits near Ciechanowice yielded the highest aggregate number of colony-forming units, but the highest levels of CFUs inside the adit were measured in the winter. The figures for the various isolated species did not differ significantly over a span of half a year; species composition, however, showed considerable variations.

The fungal species that would frequently appear regardless of the season of the year was *Cladosporium cladosporioides*, with its highest levels of CFUs in the spring: 2,800 CFUs, i.e., nearly 60% of all the recorded colonies. While the species occurred at the mouth of the adit, the interior of the whole place showed no signs of it. On the other hand, the fungus occurred in both the dark and well-lit sections during the other seasons. The growing presence of the fungus in warmer seasons, namely spring and summer, coincidencided with the periodic presence of its spores in the air [24].

The other fungus peaking in one of the seasons only was *Penicillium meleagrinum*: 1,833 CFUs were counted in the unlit section of the adit in the winter. Considering the fact that there is an amount of dead organic matter (namely rotten wood), this figure seems to correlate with the number of its spores in other underground sites [25].

All the data were also analyzed in order to assess ecological diversity. Shannon-Wiener index normally rises in response to an increase in species diversity as well as in species evenness. In most research studies concerning ecology, the index fluctuates between 1.5 and 3.5, and rarely does it exceed 4. As it is prone to subjective interpretation, the index should be weighted to allow for the variations in the environment under study. Considering the calculated Shannon-Wiener, Pielou, Simpson, and Margalef indices, it can be convincingly argued that the diversity at the investigated site was much more pronounced between the spring and the winter. Furthermore, following the Pielou index, the average distribution of species was at its most uniform in the summer, ranging between 0.812 and 0.873. This is indicative of the least noticeable dominance of any species in summer, regardless of where the sample was collected: at the entrance to the adit and in either well-lit or completely dark sections thereof.

Rozdroże Izerskie

In the case of Rozdroże Izerskie, the highest CFU count inside the adit was observed in the autumn. The diversity of the species was disproportionately lower than in the autumn, whereas the species makeup depended heavily on the season of the year.

A noteworthy feature of the adit, especially between the summer and the winter, was the pervasive presence

Table 1. CFU figures for isolated fungi species in different probing zones: Ciechanowice.

Species	Spring			Summer			Autumn			Winter		
	E	T	D	E	T	D	E	T	D	E	T	D
Acremonium strictum										40		
Alternaria alternata				73	46	113	7		20	13		
Botrytis cinerea	13			13	20		13					
Cladosporium cladosporioides	2,800			106	160	100	46	13	20	153	13	260
Cladosporium herbarum					26		13					
Epicoccum purpurascens			20									
Gliocladum catenulatum							20			33		46
Penicillium citrinum			20							7		53
Penicillium commune	20	13										
Penicillium fanthinelum					7							
Penicillium luteum		20		21	20	20						
Penicillium meleagrinum	246	680			73				180	33	20	1,833
Penicillium notatum		646		193	126		13	500	300		7	87
Penicillium purpurogenum		26		26			20		13	13		13
Penicillium urticae							14					
Penicillium vermiculatum		20										
Penicillium waxmani	113			13						13		
Sclerotinia sclerotiorium	34			21								
Trichoderma viridae							13					
Yeast-like colony					80	93	7			113		30
Non-sporulating fair colony			20	32						80	13	
Non-sporulating dark colony						53				7	7	
Sub-total	**3,226**	**1,405**	**60**	**498**	**558**	**379**	**166**	**513**	**533**	**505**	**60**	**2,322**
Grand total	**4,691***			**1,435***			**1,212***			**2,887***		
Tukey HSD Test HSD.05 = 0.41 HSD.01 = 0.99												
Margalef's Index	0.799	0.756	0.488	1.340	1.325	0.682	1.792	0.433	0.863	1.437	1.103	0.950
Simpson's Index	2.580	2.765	3.000	5.197	5.819	3.712	7.371	2.104	2.983	4.947	5.133	2.779
Shannon-Wiener Index	1.146	1.166	1.099	1.871	1.931	1.405	2.178	0.811	1.297	1.857	1.709	1.263
Pielou Index	0.551	0.599	1.000	0.812	0.838	0.873	0.908	0.585	0.667	0.807	0.954	0.575

E – Mine entrance
T – Twilight zone
D – Dark zone
Values marked with '*' significantly differ statistically

of the fungus of the genus *Penicillium*: as many as 932 colony-forming units were isolated out of the aggregate 2,372 CFUs, which constitutes approximately 39.5% of the total collected in that season.

In the autumn, on the other hand, in the unlit section of the adit, a staggering 3,440 FCUs of *Beauveria bassiana* were isolated. An impressive number of dead moths and other insets were noticed on the walls, with a growth of hyphae of entomopathogenic fungi on them, which produced a multitude of spores. The spores were observed in the water, too, where in some places dead insects floated flecked with hyphae growth. The insects most likely found

Table 2. CFU figures for isolated fungi species in different zones of probing: Rozdroże Izerskie.

Species	Spring			Summer			Autumn			Winter		
	E	T	D	E	T	D	E	T	D	E	T	D
Acremoniella atra							7		13			
Acremonium strictum							40					73
Alternaria alternata					26	6	13	20	13			30
Auerobasidium balleyi										7		
Botrytis cinerea	34									13		
Chaetomium globosum				14			7					
Cladosporium cladosporioides	106			120	67		40		14	34	453	157
Fusarium spp,										13		
Gliocladum catenulatum							30					300
Penicillium chrysogenum				7	12		13	13				
Penicillium citrinum		313	260									30
Penicillium citreo-viridae											140	560
Penicillium lilacinum							7					
Penicillium luteum				19								
Penicillium meleagrinum						5			13	110	40	
Penicillium notatum			7	46	38	11					13	26
Penicillium purpurogenum				7				13				
Penicillium waxmani				53	18	6						13
Beauveria basiana								300	3,440			
Phialophora verrucosa							4					
Populaspora polyspora									13			
Scopulariopsis brevicalis				7								
Trichoderma viridae										7		13
Yeast-like colony	87				193	132	13	30	46			127
Non-sporulating fair colony	46		113	73	7	6		46		30	73	110
Sub-total	**273**	**313**	**380**	**346**	**361**	**166**	**174**	**422**	**3,552**	**214**	**719**	**1,439**
Grand total	**966***			**873***			**4,148***			**2,372***		
Tukey HSD Test HSD.05 = 0.41 HSD.01 = 0.99												
Margalef's Index	0.202	0.000	0.179	1.248	0.787	0.900	1.574	0.513	0.613	1.136	0.464	1.128
Simpson's Index	1.582	1.000	1.054	3.721	3.663	3.596	5.634	1.319	1.039	2.800	1.842	3.311
Shannon-Wiener Index	0.554	0.000	0.121	1.599	1.439	1.335	1.912	0.535	0.124	1.404	0.831	1.503
Pielou Index	0.800	0.000	0.175	0.769	0.894	0.963	0.870	0.386	0.069	0.721	0.600	0.684

E – Mine entrance
T – Twilight zone
D – Dark zone
Values marked with '*' significantly differ statistically

their way into the adit to provide for the coming winter, which seems to account for the presence of the spores produced by the fungus. The biological diversity was at its lowest in the spring, at its highest in the summer, and medium between the autumn and the winter. It should be noted that the Shannon-Wiener index was very low in the

Table 3. CFU figures for isolated fungi species in different zones of probing: Kletno-Sztolnia Wiejąca.

Species	Spring			Summer			Autumn			Winter		
	E	T	D	E	T	D	E	T	D	E	T	D
Acremonium strictum	60	73	73									
Alternaria alternata	47				7			34	30	7		20
Auerobasidium bolleyi								13				
Aspergillus niger												7
Beauveria bassiana							60	20	379		1,406	6,013
Botrytis cinerea											13	70
Cladosporium cladosporioides		27	67		21	13		46	107		147	140
Cladosporium herbarum	80											
Epicoccum nigrum			21									
Fusarium equiseti							46		60			
Gliocladium catenulatum	80							180		30		
Humicola fuscoatra	14											
Mucor arrhizus			14									
Penicillium meleagrinum								30		7		30
Penicillium notatum		13									54	73
Penicillium paxili							120					
Penicillium purpurogenum											20	
Penicillium waksmani		7	20		12	5			13			27
Penicillium vermiculatum		20										
Phialophora verrucosa									13			
Sclerotinia sclerotiorium	55	7	13				13		13			
Trichoderma viridae									53			
Yeast-like colony	312	46	133		5	5				20	135	
Non-sporulating fair colony	67	46					146	246	87	53	80	193
Sub-total	**715**	**239**	**341**	**0**	**45**	**23**	**385**	**569**	**755**	**117**	**1,855**	**6,573**
Grand total	**1,295***			**68***			**1,709***			**8,545***		
Tukey HSD Test HSD.05 = 0.41 HSD.01 = 0.99												
Margalef's Index	0.86	1.002	0.937	0	0.542	0.346	0.726	0.865	1.706	0.529	0.54	0.799
Simpson's Index	5.172	3.213	3.925	0	2.524	1.67	2.998	2.809	2.739	1.94	1.344	1.125
Shannon-Wiener Index	1.695	1.435	1.544	0	1.004	0.591	1.274	1.363	1.402	0.846	0.553	0.314
Pielou Index	0.946	0.81	0.862	0	0.914	0.852	0.791	0.761	0.674	0.77	0.343	0.151

E – Mine entrance
T – Twilight zone
D – Dark zone
Values marked with '*' significantly differ statistically

spring, which is indicative of the low abundance of life forms and a markedly uneven distribution of species. The generally lower indices can be arguably attributed to the fact that the conditions in the adit are not favourable for the growth of various fungi, and that the presence of airborne spores is determined quantitatively and qualitatively by

Table 4. CFU figures for isolated fungi species in different zones of probing: Kletno-Sztolnia Turystyczna.

Species	Spring			Summer			Autumn			Winter		
	E	T	D	E	T	D	E	T	D	E	T	D
Acremonium murorum									47			
Acremonium strictum	60	60			1,126	526	120					706
Alternaria alternata	46		26	7			100		146			73
Auerobasidium pullulans		206								13		
Beauveria bassiana										93		
Botrytis cinerea			79							7		
Chaetomium globosum		20							13			
Cladosporium cladosporioides		186	346	13		18	80	73	233	53	26	273
Cladosporium herbarum	80		13									
Gliocladium catenulatum	80	73								80	126	573
Humicola fuscoatra	13											
Isaria farinosa					820	419						
Penicillium chrysogenum									200			
Penicillium citrinum		20								113	853	53
Penicillium expansum			20									
Penicillium implicatum									30			
Penicillium luteum												13
Penicillium meleagrinum									30			
Penicillium notatum			20						13			13
Penicillium oxalicum									13			
Penicillium paxili							1,086					
Penicillium purpurogenum		60										30
Penicillium tardum								48				
Penicillium waxmani			33		13							7
Penicillium urticae				7								
Penicillium vermiculatum			34									
Phythophthora spp,				3	3	8						
Rhizoctonia solani									26			
Sclerotinia sclerotiorium	54		13									
Scopularioppsis brevicaulis												173
Torula herbarum			20									
Trichotecium roseum												
Ulocladium botrytis									34			
Yeast-like colony	313	326	246	7	5				120	126	206	606
Non-sporulating fair colony	73	113	40	5		6	193	196	239	140	240	893
Non-sporulating dark colony							13	93	60			
Sub-total	**719**	**1,064**	**890**	**42**	**1,967**	**977**	**1,592**	**410**	**1,204**	**625**	**1,451**	**3,413**
Grand total	**2,673***			**2,986***			**3,206***			**5,489***		

Table 4. Continued.

Tukey HSD Test HSD.05 = 0.41 HSD.01 = 0.99												
Margalef's Index	1.016	0.932	1.405	0.882	0.396	0.436	0.415	0.209	1.500	1.004	0.432	1.320
Simpson's Index	5.919	4.323	2.797	3.261	1.984	2.083	1.587	1.918	5.053	4.826	1.448	4.029
Shannon-Wiener Index	1.841	1.648	1.545	1.272	0.726	0.808	0.757	0.672	1.892	1.690	0.622	1.661
Pielou Index	0.946	0.847	0.671	0.917	0.524	0.583	0.546	0.969	0.789	0.868	0.449	0.693

E – Mine entrance
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Values marked with '*' significantly differ statistically

the water level, which changes every season. It seems reasonable to assume that the vernal and summery surge of water brings organic matter, which boosts fungi growth and fosters their biological diversity.

Kletno: Sztolnia Wiejąca and Sztolnia Turystyczna

The adits have been matched against each other because they are within a fairly short distance of each other, but the two house surprisingly disparate collections of fungal species, which seems incontrovertibly connected with tourist traffic. Only 24 fungal species were identified in the adit that is not a tourist attraction and 35 in the tourist adit. The highest number of CFUs were isolated in the winter: 8,545 in Sztolnia Wiejąca and 5,489 in Sztolnia Turystyczna.

In both adits, dead insects flecked with entomopathogenic fungi hyphae growth were widely found. In the air, however, the spores of many other species were identified. The air in Sztolnia Wiejąca, especially in winter, was infested with *Beauveria bassiana* spores, which constituted around 87% of all identified spores. The *Beauveria bassiana* spores were also in profusion in the autumn, accounting for approximately 27% of all spores in the air under study. The *Beauveria bassiana* fungus was also isolated in Sztolnia Turystyczna, but nonetheless in considerably lower numbers. In the summer, 1,239 CFUs of another entomopathogenic fungus, *Isaria farinosa* were identified in the adit.

Sztolnia Turystyczna provided suitable habitat for *Acremonium strictum*: a fungus that was within a range of around 4.5% in the spring to a handsome 55.5% of the total count of spores in the summer. The fungus is a saprotroph commonly occurring in soil, in decaying plants, and mushrooms. Interestingly, in rare instances the fungus can turn pathogenic to humans [27].

An obvious correlation between tourist traffic and the multifarious life forms and their balanced occurrence at the site are clearly revealed through analysis of the biodiversity indices. Sztolnia Turystyczna is fundamentally distinct in species evenness, particularly from summer to winter. Generally higher Margalef and Simpson indices, too, point irrefutably to the much richer variety of the isolated fungi at the site widely accessible to the public, which is corroborated by similar independent studies [28-29].

General Discussion

It seems advisable to consider the very count of airborne spores in one cubic meter. Porca et al., in "Aerobiology: An ecological indicator for early detection and control of fungal outbreaks in caves" (2011), offered a broad classification of caves into five categories (depending on CFU/m^3) in order to establish whether the presence of airborne fungi exerts a negative impact on the environment of the site under study and whether it would be recommendable to impose some restrictions on tourist traffic and to monitor air flow as well as to possibly keep it under control by means of airlocks or gates [8].

Following this classification, each site discussed in our paper – except Rozdroże Izerskie between spring and summer, and Sztolnia Wiejąca in summer – would fall into category No. 5, and as such would become eligible to be granted a "red alert" status: an irreversible disruption to

Fig. 1. Locations of research sites.

ecological balance. Our research demonstrated that the CFU/m^3 figures for fungal spores exceeded substantially those disclosed by Porca et al. Taking into account Porca's classification, it is nevertheless therefore desirable to contemplate such factors as the environmental diversity of the investigated sites and the enormously contributing elements, e.g., the presence of organic matter or accessibility to animals (which can be seasonal visitors) [30].

Threshold values proposed for Porca's classification become profoundly important in the case of underground sites where the fungal presence may materially affect, for instance, hardly noticeable but historically significant features such as cave paintings [31-32]. The classification should then incorporate additional elements such as historical relics or artefacts/man-made structures that might be susceptible to the presence and effect of micro-organisms, including fungi. It is noteworthy that three out of four sites under study are not officially listed as tourist spots and access to them as such is by no means regulated.

Conclusions

Underground sites provide habitat for unique flora and fauna. The seasonal occurrence of some organisms, such as insects and mammals (e.g., bats [11]), have a far-reaching effect on the annual species variation in microflora. The collection and composition of airborne fungi varies depending on the site under study: there was a more profound difference between the adits inaccessible to tourists and the tourist adit at Sztolnia Turystyczna. The presence of tourists has a massive impact on fungi in terms of species composition as well as the number of the specimens and spores in these places, which was demonstrated by the verified biodiversity indices. This may have a tremendous influence on the stability and maintenance of places in terms of historical continuity [12, 33]. The study calls for continuation while the methods employed in sample collection and analysis need to be enhanced.

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