M. Rdzanek, W. Pusz, E. Gębarowska, and E. Pląskowska – Airborne bacteria and fungi in a coal mine in Poland. *Journal of Cave and Karst Studies*, v. 77, no. 3, p. 177–182, DOI: 10.4311/2015MB0102

AIRBORNE BACTERIA AND FUNGI IN A COAL MINE IN POLAND

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Abstract: We determined the species composition and the concentration of colony forming units of airborne bacteria and fungi in a coal mine in Poland. We sampled at six locations in a working shaft at about 500-600 m below ground level. Air samples were collected between 6 and 9 a.m. using the impact method onto Potato Dextrose Agar and TSA. The volume of air filtered for each coalmine sample was 50 L. We found 11 fungal species, with *Penicillium meleagrinum* and *P. notatum* as the most common. Six bacterial genera were identified, with *Micrococcus* spp. as the most common. There were no pathogenic fungi or bacteria identified. The concentration of fungal spores and bacteria in the coal mine air is not a direct hazard to mine workers.

Introduction

Bioaerosols are ubiquitous in indoor air and may be potentially negative to human health. Bioaerosols or organic dust may include pathogenic or non-pathogenic live or dead bacteria and fungi, viruses, high molecular weight allergens, bacterial endotoxins, mycotoxins, peptidoglycans, β(1→3)-glucans, pollen, and plant fibers. Bioaerosols are transmitted by the airborne droplets or dust through the skin, mucous membranes, and respiratory tract and rarely get into animals and humans orally (European Agency for Safety and Health at Work, 2000; Douwes et al., 2003; Górny 2004). Air contaminated with bioaerosols can be an important source of infection for humans. Depending on the composition of bioaerosols, they can cause simple irritation or ailments, allergic reactions, light or serious infections, and toxic reactions (Douwes et al., 2003; Gamboa et al. 1996; Górny 2004). Therefore, the concentrations of individual components of bioareosols measured by CFU of bacteria and fungi is one of the indicators of indoor air pollution (Douwes et al., 2003; European Agency for Safety and Health at Work, 2000; Drenda, 2012).

Working conditions in coal mines are hard, due to the depths of the mines, which can be up to 1000 m, high temperatures reaching over 30 °C, and relative humidities of 70 to 100% (Drenda, 2012). These conditions, plus the presence of organic substances, create a fairly favorable growth environment for microscopic fungi, which can be found on any organic material used in the mining process, especially on timber, all types of organic waste, as well as on insulators, machinery tires, or other rubber surfaces (Piontek and Bednar 2010). The miners going down to work bring fungi into the mine. The research conducted by Pusz et al. (2014) in a copper mine has shown that the number of colony forming units (CFU) is dependent on the kind of work done and presence of organic material.

The level of bioaerosols found underground in coal mine shafts was high, containing a great number of spores spread through the shafts by the ventilation system. Frequent air changes, which are characteristic in the microclimate of the mine, result in significant variations of the number of CFU present in the air samples. Some of the airborne fungal species (*Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp.) that occur in the mine excavations and galleries can possibly cause individual allergies and fungal infections in some exposed workers (Gamboa et al. 1996; Obtułowicz 2006; Cabral 2010).

The official limits of indoor airborne bacterial and fungal spores concentrations used in Poland as safety standards (Polish Committee for Measurements and Quality Standards 1989a, 1989b) do not apply to mine excavations or galleries. Most species of the fungi spores found in the coal mine air samples and on organic material, such as timber safety structures built in the galleries, can produce mycotoxins that can cause different diseases when introduced into the human body (Rusca et al. 2008).

In Poland, there are no legal regulations that would allow for a reliable assessment of the microbiological quality of air (Tsapko, et al. 2011). The reference point is the European Agency for Safety and Health at Work (2000) and the proposed limit values given in the literature (e.g., Górny 2004, 2010). The aim of our research was to determine which species of airborne bacteria and fungi occur in the coal mine and to identify their CFU concentration.

Materials and Methods

This study was performed in May 2014 in the coal mine KWK Murcki-Staszic, owned by Katowicki Holding Węglowy S.A. The coal mine is located in Katowice, Upper Silesia, south Poland (Fig. 1). The mine under its present name was formed on 1 January 2010 as the result of merger of the oldest, KWK Murcki, and one of the youngest, KWK Staszic, mines in Upper Silesia. The mine underlies more than 67 km^2. Extraction is carried out at five levels from

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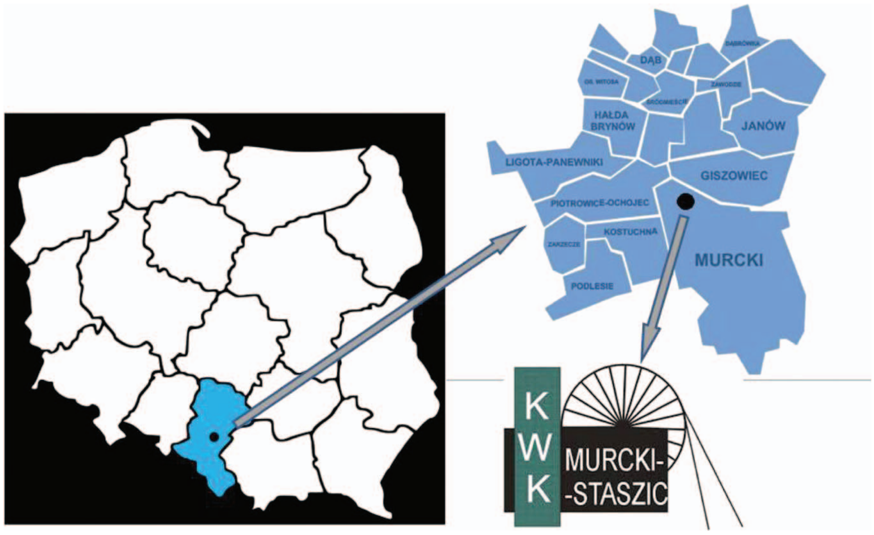

Figure 1. Location of the KWK Murcki-Staszic coal mine in Poland.

416 to 900 m below ground level. Daily production of the mine is an average of 23,000 tons. The mine's coal resources are estimated at about 50 years of extraction.

Air measurements were taken at six sampling points in the working shafts at depths of 500 and 600 meters below ground level. The sample locations are referred to as *shaft insert*, the bridge at the mouth of the mineshaft from which the crew enters the mine cage to go down to the mining level; *shaft bottom*, an underground excavation at the mining level of 500 m directly below the mine shaft, the place where the crew leaves the mine cage; *shaft collar*, a ventilation shaft located a few hundred meters from the main shaft through which the exhaust air leaves the mine; *miner's meeting place*, a small chamber located a few hundred meters from the shaft bottom where workers gather before starting work, from which the miners' brigades move forwards to their workplaces; *working face*, the surface at the end of the mining corridor about 1000 m from the shaft bottom where the mining work is advancing, with a machine and a crew of about ten men and a very high dust level; *station to operate machines*, a miners' work station located in the middle of the corridor with a conveyor belt that transports the excavated material.

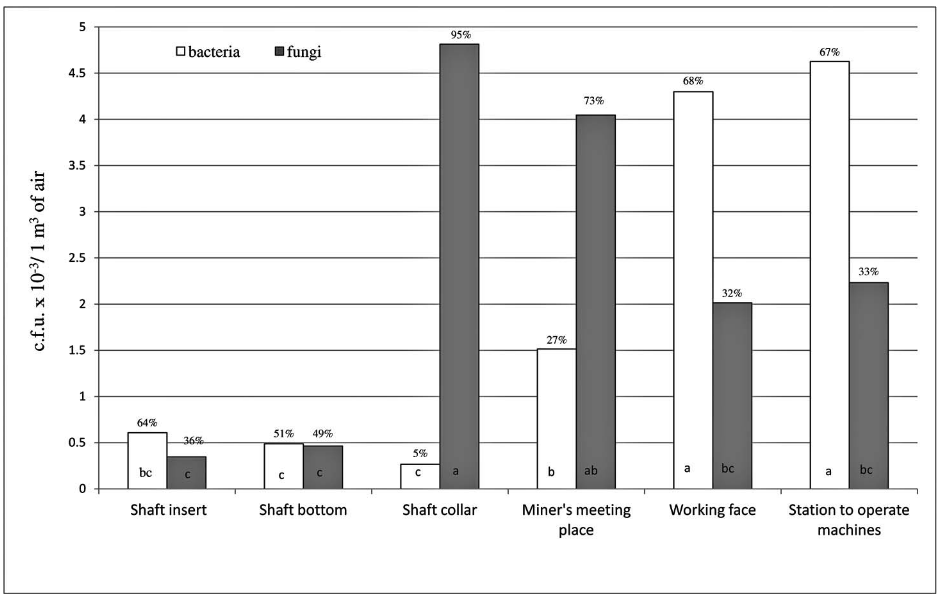

Figure 2. Measured concentrations of bacteria and fungi in the air at the samples sites. Percentages are of the total microorganisms at the site. Bars marked with the same small letter a, b, or c did not differ significantly ($p < 0.05$) by the Tukey test.

Table 1. The total concentrations of microorganisms in the air by sample location.

Elements of Bioaerosol	Shaft Inset	Shaft Bottom	Shaft Collar	Miner's Meeting Place	Working Face	Station to Operate Machines
The total number of fungi (CFU/m^3 of air)	347	463	4813	4047	2013	2233
The total number of bacteria (CFU/m^3 of air)	607	487	267	1513	4300	4627
The number of bacteria and fungi together	954	950	5080	5560	6313	6860

The samples were collected between 6:00 and 9:00 a.m., when 200 miners were working in the mine. We used the impact method and the Air Ideal 3P sampler to analyze the fungal load using PDA (Potato Dextrose Agar) plates manufactured by Biocorp. For the isolation of bacteria, TSA medium was used (Trypcase-Soy Agar by bioMerieux, France). In order to eliminate fungi and yeast from the bacteria samples, 30 g/mL of nystatin was added to the TSA medium (Polfa, Kraków).

The volume of each collected coal mine-air sample was 50 L, 100 L, or 150 L. The sampling at each site was performed three times, and the impactor was held at 1.5 m above the floor. The PDA plates were incubated for 7 days at room temperature (22 °C). The TSA plates were incubated for 72 hours at 37 °C. After incubation the number of visible colonies was determined, and the fungi were identified to species according to their morphology. Pure cultures of bacteria were obtained by reductive isolation and then diagnosed by Gram staining, spore staining, catalase test, and API tests (BioMerieux, France). The number of colony forming units (CFU) per 1000 L (1 m^3) of air was determined by $X = (a \times 1000)/V$, where a is the total number of colonies grown on three plates and V is the collected air volume of 150 liters (50 L for each plate).

The resulting concentrations of microorganisms per 1 m^3 of air (CFU/m^3) were compared with the guidelines developed by the Team of Experts of Biological Factors (ZECB) (Górny, 2004 and 2010). The results obtained from the total numbers of bacteria and fungi (CFU/m^3) were also subjected to analysis of variance using the Tukey test ($p < 0.05$) using Statistica for Windows v. 5.1.

Results

The numbers of bacteria and fungi present in the tested air are shown in Figure 2 and Table 1. The presence of bacteria and fungi at the sampling points varied. The number of bacteria developed in the range of 0.27 to 4.63 × 10^3 CFU/m^3 of air, and fungi from 0.35 to 4.81 × 10^3 CFU/m^3 of air. The highest concentration of bacteria (above 4.3 × 10^3 CFU/m^3) was observed at underground depths of 500 to 600 m, at the station to operate machines and the working face. At these sample points bacteria were the predominant microflora in the bioaerosol (~ 68%). The lowest number of bacteria (0.27 × 10^3 CFU/m^3) were isolated from the shaft collar. However, at this point the air was contaminated with fungi (4.8 × 10^3 CFU/m^3); they accounted for 95% of all microorganisms. Similarly, a high number of fungi in the air was observed at the miner's meeting place (above 4.0 × 10^3 CFU/m^3). The lowest counts of both groups of microorganisms were observed in the bioaerosol in the shaft insert and shaft bottom. The number of fungi and bacteria in these samples did not exceed 6.1 × 10^2 CFU/m^3 of air. These measuring points are in open spaces, which usually have a

Table 2. Concentrations in CFU/m^3 of types of bacteria in the air at sampled sites, with percentages of all bacteria at the site in parentheses.

Species	Shaft Inset	Shaft Bottom	Shaft Collar	Miner's Meeting Place	Working Face	Station to Operate Machines
Micrococcus spp.	287 (47)	227 (47)	127 (47)	840 (56)	2780 (65)	3187 (70)
M. luteus	67 (11)	60 (12)	53 (20)	340 (22)	660 (15)	360 (8)
Gram-positive cocci	nd	nd	nd	73 (< 5)	60 (1)	140 (3)
Staphylococcus spp.	93 (15)	67 (< 14)	20 (7)	27 (2)	180 (4)	280 (6)
S. epidermidis	7 (1)	7 (1)	20 (2)	33 (2)	167 (< 4)	80 (< 2)
S. equorum	13 (2)	nd	nd	nd	nd	nd
S. hominis	nd	nd	nd	20 (1)	127 (< 3)	153 (3)
S. saprophyticus	40 (< 7)	33 (< 7)	7 (2)	73 (< 5)	200 (< 5)	227 (5)
S. xylosus	nd	nd	nd	13 (< 1)	93 (2)	107 (2)
Gram-negative rods	73 (12)	40 (8)	47 (17)	40 (< 3)	nd	13 (< 1)
Bacillus spp.	27 (4)	20 (4)	7 (2)	40 (< 3)	nd	7 (< 1)
Actinomycetes	nd	33 (< 7)	nd	13 (< 1)	33 (< 1)	73 (< 2)

Note: nd = not detected.

Table 3. Concentrations in CFU/m^3 of types of fungi in the air at sampled sites, with percentages of all fungi at the site in parentheses.

Species	Shaft Inset	Shaft Bottom	Shaft Collar	Miner's Meeting Place	Working Face	Station to Operate Machines
Aspergillus niger Tiegh.	7 (2)	nd	nd	nd	nd	nd
Aspergillus versicolor (Vuill.) Tirab.	nd	nd	nd	33 (< 1)	30 (1)	113 (5)
Cladosporium cladosporioides (Fresen) G.A. de Vries	nd	13 (3)	nd	nd	nd	nd
Mucor flavus Schrank	nd	nd	nd	nd	7 (< 1)	nd
Mucor hiemalis Wehmer	10 (3)	nd	nd	nd	nd	23 (1)
Penicillium chrysogenum Thom	nd	57 (12)	nd	nd	nd	nd
Penicillium meleagrinum Biourge	60 (17)	230 (50)	2890 (60)	687 (17)	1943 (97)	1740 (78)
Penicillium notatum Westling	150 (43)	43 (9)	1887 (39)	3317 (82)	33 (< 2)	313 (14)
Talaromyces funiculosus (Thom) Samson, N. Yilmaz, Firsvad & Seifert	120 (35)	50 (11)	37 (< 1)	10 (< 1)	nd	nd
Trichoderma hamatum (Bonard.) Bainier	nd	nd	nd	nd	nd	43 (2)
Trichoderma harzianum Rifai	nd	70 (15)	nd	nd	nd	nd

Note: nd = not detected.

smaller amount of microorganisms. Contributions of individual genera and species of bacteria in the tested air are shown in Table 2. The dominant group of bacteria (from 49% to 80%) were Gram-positive cocci, especially of the genus *Micrococcus* spp. We also isolated staphylococci, the most numerous of those species being *Staphylococcus saprophyticus* and *S. epidermidis*.

Actinobacteria were also present in the coal-mine samples, with their greatest concentration observed in the station to operate machines (7.3×10^1 CFU/m^3). However, the frequency of their occurrence in comparison to the total number of bacteria was very low. Their share in the total number of bacteria did not exceed 2%. We did not identify pathogenic Gram positive or Gram negative bacteria in the air.

The contributions of individual species of fungi in the air of the mine are shown in Table 3. The largest share of fungi were of the genus *Penicillium*, especially *P. notatum* and *P. meleagrinum*.

Discussion

The presence of microorganisms in underground halls and corridors is correlated with the number of users, the relative humidity, and the amount of dust particles. The number of bacteria in such areas can be up to several thousand per m^3 of air (Błaszczyk, 2010, p. 357–369). It is assumed that the total number of bacteria and fungi combined in manufacturing and industrial areas should not exceed 1×10^7 CFU/m^3 of air. Recommendations of the Zespoół Ekspertoów ds. Czynnikoów Biologicznych (Team of Experts of Biological Factors) was used as the norm for mesophilic bacteria of 1×10^5 CFU/m^3, for gram-negative 2×10^4 CFU/m^3, and for total fungi 5×10^4 CFU/m^3 (Górny 2004, 2010). Measured concentrations of microorganisms at all of the measuring points were within acceptable values according to Górny (2010).

None of the examined sites exceeded normative values for Gram-negative bacteria. This type of bacteria was isolated at less than 1×10^2 CFU/m^3 of air in the production halls. The standard is 1×10^3 CFU/m^3 according to Górny (2010). *Actinomycetes* are bacteria commonly present in soil. They are also detected in air samples and are common environmental pollutants in production and housing spaces. Occurrence of them indoors is associated with increased humidity (Frączek and Kozdrój, 2013). The highest concentration of those airborne microorganisms was recorded in the station to operate machines (7.3×10^1 CFU/m^3). According to the recommendations of the PN-89 Z-04111/02 and presented in it, the scale of actinobacterial air pollution at this location was moderate (Górny 2010). Frączek and Kozdrój (2013) conduced similar experiments in a subterranean spa located in the former salt-mine of Bochnia. They report the average concentrations of actinobacteria underground ranged from 0 to almost 4×10^1 CFU/m^3 throughout the year.

Microorganisms commonly found in the air are natural components of the bacterial flora of the skin and mucous membranes. Responses of individuals to mine exposures to bioaerosols depend in their individual sensitivities, including immunity, time of exposure, or dose of microorganisms introduced into the body. They can cause immunopathogenic reactions (Szczuka et al., 2013). Inhalation of bioaerosols, depending on the composition, exposure time, and sensitivity of the human immune system, can cause respiratory disorders such as allergic rhinitis, asthma, allergic alveolitis, bronchitis, chronic pulmonary insufficiency, tuberculosis, sinusitis, or conjunctivitis (Gąska-Jędruch and Dudzińska, 2009; Prasanth et al., 2011). Piontek and Bednar (2010) claim that *Penicillium* spp., *Aspergillus* spp., and other frequently isolated fungi, if present in sufficiently large quantities, can damage the mining machinery, timber structures, or cables, and as a consequence, result in serious accidents.

Unlike other working environments, there is little research on the concentration of bacteria and fungi in the halls of underground coal mines. Previous work describes exposure to bioaerosols in the chambers of the Wieliczka salt mine and copper mine in Lubin. Pusz et al. (2014) studied the occurrence of air-borne fungi in three copper-mining shafts: Bolesław, Lubin Zachodni (Lubin West shaft), and Lubin Główny (Lubin Main shaft) in the Lubin mining site, property of KGHM Polska Miedź S.A. They found twenty-seven fungal species, the most numerous being *Penicillium notatum*, *P. urticae*, and *Aspergillus flavus*. The population of fungi varied considerably among the copper mines' shafts or shaft parts. The maximum concentrations ranged from 5.06×10^3 CFU/m^3 of air in Lubin Main shaft to 2.15×10^4 CFU/m^3 in Bolesław shaft. The largest concentration of spores was observed in the part of mine where the timber was stored (2.15×10^4 CFU/m^3). The KWK Murcki-Staszic coal mine was also dominated by fungi of the genus Penicillium (*P. notatum* and *P. meleagrinum*), but their concentration in the air was much lower. That could be caused by a smaller amount of organic matter present in the mine and a much lower prevailing temperature in the coal mine. In the studied mine, timber was not stored underground, and the place where the highest concentration of fungi recorded was the shaft collar (4.81×10^3 CFU/m^3). Ogórek (2012) examined air quality in a gold mine in Złoty Stok (Poland), which turned out to host several fungal species, with *Trichoderma harzianum*, *P. expansum*, and *Botrytis cinerea* being the most numerous. *Aspergillus* and *Penicillium* are capable of producing mycotoxins, which are hazardous to human health (Cabral, 2010), and the spore concentration of those fungi in the gold mine examined by Ogórek (2012) could be a health threat to the miners working in some parts of it. Again the highest concentration of spores was present in the parts of mine abundant in timber.

Prasanth et al. (2011), in his work on a lignite opencast mine in India, describes the occurrence of fungi. They recorded a total of 520 colonies of fungi with an average of 260 CFU/m^3 of air. The isolated colonies were classified into 24 species belonging to 13 genera of fungi. Among the species recorded, *Aspergillus niger* was dominant in the atmosphere, with 82.5 CFU/m^3 of air and *Penicillium restrictum* (21.5 CFU/m^3 of air). Spore concentration was much lower than in the case of KWK Murcki-Staszic and species composition was more varied, which could be due to the different characters of open and underground mines. The air indoors is more polluted than outdoors. The degree of contamination depends on the premises, number of people, ventilation, and outdoor-air properties.

Much more research is concerned with the quality of air in caves than in active mines because of the frequency of tourist visits, which makes it necessary to know about possible potentially pathogenic microorganisms in caves, determine their reservoirs, and inform the public about the consequences of such visits (Jurado et al., 2010). The concentration of spores and bacteria in natural caves are usually much lower than in the mines. For example, in a Niedźwiedzia Cave in the Sudety moutain, which is often visited by tourists, Ogórek et al. (2014a) reported the concentration of spores between 123 and 214 CFU/m^3, while the most commonly isolated fungi were *Cladosporium herbarium* and *Rhizopus stolonifer*. Similar results were obtained by Ogórek et al. (2014b) examining the air in an artificial underground complex named Włodarz, located inside the massif of Włodarz, within the Owl Mountains, Lower Silesia, Poland. Between 65.5 and 1003 colony-forming units of fungi per m^3 of air were isolated from the air sampled in the adit, and the most common fungus isolated from the air outside and inside the adit was *Cladosporium cladosporioides*, followed by *C. herbarum* at one location in the adit.

On the other hand, Pusz et al. (2015) isolated from the newly discovered cave Jarkowicka, visited so far by few people, 22 species of fungi, whose concentration in the air, depending on the measure point, ranged from 76 to 200 CFU/m^3. *Cladosporium cladosporioides* was the fungus most frequently isolated. It amounted to almost 75% of air-borne spores inside the cave. *Alternaria alternata* spores were also relatively frequent in the cave, making up 12% at the entrance and 9% in the middle section. The reason for such large differences in species composition and concentration of fungi in bioaerosols may be differences in air temperature, relative humidity, and the presence or absence of organic matter in each area.

Conclusions

The number of fungi and bacteria isolated from the tested air is not a threat to the health of people working in the coal mine KWK Murcki-Staszic. The microbiological quality of indoor underground air is connected, in this case, primarily with the effectiveness of the ventilation system. Modernization of the existing ventilation system and the introduction of modern, better equipment can improve aerosanitary conditions in the underground mine.

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