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Determination and Statistical Analyses of the Total Microfungal Flora of a Coal Mine in Manisa

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Abstract
The aim of our study is to determine and minimise the fungus related health problems for mine workers by determining the potential pathogenic microfungi in the outdoor and indoor air of an underground coal minery. For this reason air samples were taken from 42 sampling sites of 6 stations including main carriage canals, carriage canals and desandre canals, mechanised areas in which the production is held by machinery, manual areas in which the production is held by mine workers, the ceiling areas and areas that are outside of the mine and Merck Mas 100 Eco Air Sampler, with an airflow rate of 100 L/min, was used. A total of 11959 colonies were detected and 10 genera and 25 different species were isolated. The results were given in cfu/m3. The genera identified were *Aspergillus, Alternaria, Amorphotheca, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, Scopulariopsis, Trichoderma* and mycelia sterilia.
Keyword: Coal mine, microfungi, potentially pathogenic, isolation, identification

INTRODUCTION

Fungi, which reserve an important place in the study of microbiology, are distributed on all af the spheres of the earth and are abundantly found in air [1].

The airborne fungi are one of the most commonly seen organisms in nature [2]. The concentration of airborne fungal spores is affected from many of the environmental factors such as humidity and temperature and various specific contamination sources [3]. It is known that besides being related to air pollution, fungi may have some negative effects on human, animal and plant health and that they may cause health problems on humans via infections, hypersensitivity reactions and toxic reactions [2 – 6]. Recent studies showed that health problems such as respiratory track infections, bronchitis, asthma, immune system disorders and fatigue are likely to be seen in people who are working in places which are damp and microfungally rich [7]. It is reported that the presence of 10^3 cfu/ m^3 microfungi in indoor air may cause extraordinary health situations [4].

Unlike other microbial pathogens, some fungi such as *Aspergillus, Alternaria, Fusarium* and *Mucor* can cause opportunistic infections, and so they are also a great risk for patients with suppressed immune system such as AIDS, diabetes, organ transplantation and cancer patients [8–11]. Many of the fungi belonging to *Aspergillus, Alternaria, Cladosporium, Fusarium, Penicillium, Trichoderma* and *Epicoccum* genera are known to cause immune pathologic disorders such as Allergic Fungal Sinusitis, Allergic Bronchopulmonary Mycosis, Allergic Bronchopulmonary Aspergillosis [8, 12–14]. Besides these, inhalation of mycotoxins and other secondary metabolites released from fungal spores and colonies, can cause many disorders including irritation of the mucose membranes, nausea, immune system deficiency, acute or chronic damages in liver and central nervous system and cancer [15].

As one of the major raw material of Turkey's energy demand, lignite has a production value of 52 million tons and 10% of this production is carried out in underground coal mines since some of the coal formations are under the thick upper layer [16]. Workers who work in the underground coal mines spend most of their time in indoor environments and it is known that in environments which are damp, humid and not well aerated, the complaints of allergies and other fungal related diseases tend to increase [17]. Besides, the various wooden construction materials used in the mines and the surfaces of deposited mines also create convenient environments for potentially allergenic molds. Even though the lung diseases which are related to coal dust, such as pneumoconiosis, silicosis, emphysema and chronic bronchitis are more likely to be seen in coal mine workers, these diseases pave the way for mold related diseases like aspergillosis [18–19].

Besides the dust particles, miners who work in underground systems also inhale the microflora attached to these particles and the microfungus spores ($\leq 5\mu M$) that are found freely in air, and the inhalation of these spores and microorganisms may potentially lead to evaluable health problems by producing mycotoxins and allergenic proteins.

In Turkey and throughout the world, the studies of airborne potentially allergenic microfungi have come into prominence in recent years and most of these are about determining indoor and outdoor air quality, the potentially allergenic species, their seasonal distribution, and their effects on humans [20 – 39].

Scarcely there are no studies about the airborne potentially pathogenic microfungi in the indoor and outdoor air of underground coal mines held in our country. Considering the miners that work in the underground mines; the temperature, humidity and oxygen levels of the

mines are measured on a regular basis and fixed to a certain level. In accordance with the Bylaw on Occupational Health and Safety Measures in Mines and Quarry Enterprises and Tunnel Construction no. 18553 of 22.10.1984, it is prohibited to work in areas where concentration of oxygen is below 19% or concentration of carbon dioxide is over 0,5%. The same bylaw stipulates that temperature should not below 8°C and over 30°C. Although steel construction has been used in recent years for interior fittings of mines, wooden fittings are still used and they constitute a convenient environment for colonization of microfungi within limits stipulated in the bylaw. Whether mechanically or manually performed all of the methods used in production generate dust and this dust disperses through the whole mine by the effect of air circulation.

For all those reasons designated above, this study is aimed to assess the total values of microfungi, determine the airborne potentially pathogenic microfungi by isolating and identifying the isolates in species level in and outdoor air of a underground coal mine and specify the risks that coal miners work there.

MATERIALS AND METHODS

The study area

The study took place in an Underground Coal Mine in Soma, Manisa in Turkey.

According to the official website of Manisa, "Despite being landlocked, Manisa is the closest city to the shore in Western Anatolia. Batı It is located between 27 08' – 29 05' eastern longitudes and 38 04' – 39 58' Northern latitudes. It is surrounded with Usak and Kutahya from east; Izmir from West; Balikesir from North; Aydin from South and Denizli from Southeast. The surface area of the is 13.810 km^2. The altitude varies between 50 -850 m and the elevation rises eastward from provincial center. Both Mediterranean climate and continental climate of central Anatolia region can be seen in Manisa. While Mediterranean climate is seen on coombs and valleys; in highlands, platoleaus and mountains the effects of continental climate is seen. Summers are considerably hot and the average temperature is 17.5^{o}C. The precipitation properties of western Anatolia is the same of Mediterranean climate type. While the precipitations are commonly seen in winter months, summers are droughty. The average amount of rainfall is 713.6 kg. The 46 % of provincial Manisa land is covered with maquis and forests. Forests consist of oak (*Quercus sp.*), ash tree (*Fraxinus sp.*), elm tree (*Ulmus sp.*), larch (*Pinus nigra*), turkish pine (*Pinus brutia*), Juniper (*Juniperus sp.*), wild pear (*Pyrus elaeagrifolia*) and sycamore (*Platanus sp.*) trees. Forests have an extensive coverage [www.manisa.bel.tr].

The experimental design

Pursuant to Bylaw on Occupational Health and Safety Measures in Mines and Quarry Enterprises and Tunnel Construction no. 18553 of 22.10.1984, which stipulates the definition of and conditions for mines; "Mine is a work environment that involves shafts and routes of access and exit as well as all excavations underground; isolated and straight galleries where tallows from these excavations are removed; other routes and production locations; excavation (extraction), transportation, ventilation plants; and permanent facilities that are used for provision and transmission of energy used underground. Every mine with a specific ventilation plant is considered to be an independent mine; while multiple mines owned by the same employer, which are centrally managed and interconnected underground, are considered to be a simple mine." [40]. The construction plan of the mine in which the study held is shown in Figure 1.

Merck MAS 100 Eco was used for air sampling and the sampling took place between 10:00 – 14:00 pm. The sampling device was held 1,40 – 1,50 m above the ground level to be conformed with the breathing height of humans while sampling. The lid of the device was disinfected with 70% ethanol before every usage. The petri dishes containing sterile Dichloran Rose–Bengal Chloramphenicol Agar (Merck 1.00466) were placed into the air Sampler and 100 liters of air were taken to each petri dish. Air samples were taken from 42 sampling sites of 6 stations including main carriage canals, carriage canals and desandre canals, mechanized areas in which the production is held by machinery, manual areas in which the production is held by mine workers, the ceiling areas and areas that are outside of the mine (Table 1). Petri dishes containing the air samples were incubated 5 – 7 days at 25°C and after the incubation period, the colonies counted were inoculated to Malt Extract Agar (Merck 1.05398) and Potato Dextrose Agar (Merck 1.10130) slants for isolation. After incubation the slants were retained at 4°C for identification. The number of fungi counted were evaluated as cfu/m^3[17].

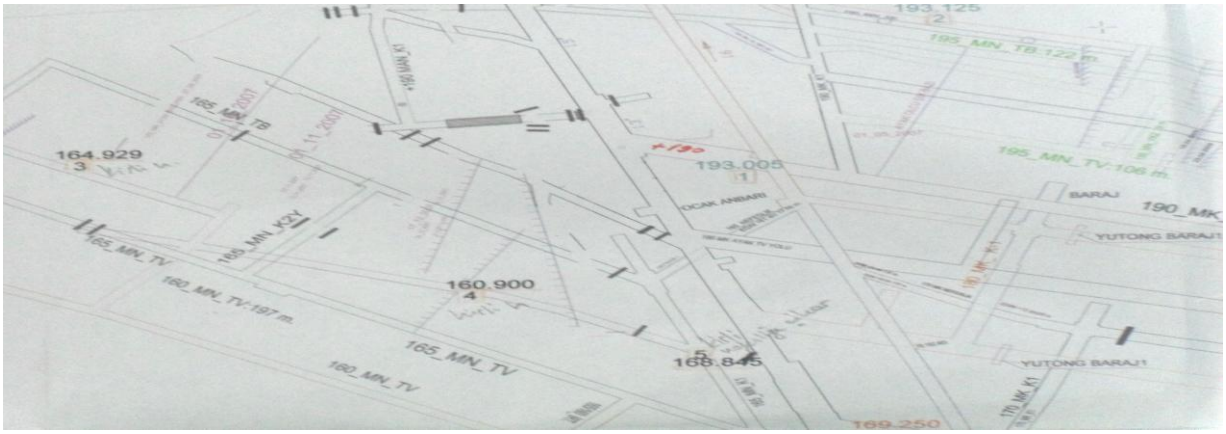

Figure 1. The construction plan of the minery in which the study held

Table 1. The stations, Sampling Points and the temperatures degrees of the sampling points

Stations	Sampling Point No	Sampling Points	Temperature (°C)
Main Carriage Canals	1	main carriage canals 1	26,1
	2	main carriage canals 2	23.1
	3	main carriage canals 3	24,4
	4	main carriage canals 4	21,2
Manual Areas	5	190 Manual 1	23,9
	6	190 Manual 2	23,8
	7	190 Manual 3	24,0
	8	190 Manual 4	24,1
	9	B12 Manual Base 1	24,0
	10	B12 Manual Base 2	22,4
	11	B12 Manual Base 3	22,9
	12	160 Manual 1	23,8
	13	160 Manual 2	25,1
	14	160 Manual 3	24,6
	15	160 Manual 4	23,8
	16	160 Manual 5	23,7
Ceiling Areas	17	170 Ceiling 1	24,0
	18	170 Ceiling 2	23,3
	19	170 Ceiling 3	23,5
	20	170 Ceiling 4	23,9
Desandre canals	21	Desandre 1	24,5
	22	Desandre 2	23,6
	23	Desandre 3	24,2
Carriage Canals	24	Main Carriage 1	22,3
	25	Main Carriage 2	22,8
	26	Main Carriage 3	23,3
	27	Main Carriage 4	23,1
	28	Main Carriage 5	23,8
	29	170 Carriage Canal 1	23,3
	30	170 Carriage Canal 2	23,7
Main desandre canals	31	Main Desandre 1	24,1
	32	Main Desandre 2	24,0
	33	Main Desandre 3	23,7
Mechanised Areas	34	B1 Mechanised1	23,9
	35	B1 Mechanised2	22,5
	36	B1 Mechanised3	22,3
	37	B1 Mechanised4	23,3
Outdoor areas	38	air well	32,5
	39	Ground Floor	29,3
	40	Entrance of coal mine	30,4
	41	Managers Office	25,2
	42	Entrance of administrative personnel building	29,4

For identification at genus level, the colonies isolated were inoculated to petri dishes containing Malt Extract Agar and Potato Dextrose Agar and incubated at 25°C for 7 days. The colonies were examined microscopically and macroscopically and Barnett (1998), Hasenekoglu (1991) and Domsch et al (1980) were used for identification [41 – 43].

Species level identification of genus *Aspergillus* was based on "Identification of Common *Aspergillus* Species (Klich, 2002)" and as identification media CYA25 (Czapeks Yeast Extract Agar incubated at 25 °C), CYA37 (Czapeks Yeast Extract Agar incubated at 37 °C), MEA are usually found on altitudes higher than 1000m. the 39.1 % of provincial land is planted areas and vineyards and olive groves (Malt Extract Agar), CY20S (Czapeks Yeast Extract Agar with 20 % Sucrose) and CZ (Czapeks Dox Agar) were used. Isolates inoculated to these media, were incubated at 25°C for 7 days and after incubation they were macro- and microscopically examined [44].

Species level identification of genus *Penicillium* was based on "A Laboratory Guide to Common *Penicillium* Species (Pitt, 2000)" and as identification media CYA25 (Czapeks Yeast Extract Agar incubated at 25 °C), CYA37 (Czapeks Yeast Extract Agar incubated at 37 °C), CYA5 (Czapeks Yeast Extract Agar incubated at 25 °C), MEA (Malt Extract Agar), G25N (Glycerol 25% Nitrate Agar) and CSN (Creatine Sucrose Agar) were used. Isolates inoculated to these media, were incubated at 25°C, 37°C and 5°C for 7 days and after incubation they were macro- and microscopically examined [45].

For other genera "Soil Microfungi (Hasenekoğlu, 1991)", "Illustrated Genera of Imperfect Fungi (Barnett 1998)" and "Compendum of Soil Fungi (Domsch et al., 1995)" were used and isolates were inoculated to PDA (Potato Dextrose Agar) and MEA)Malt Extract Agar). After incubation at 25°C for 7 days isolates were macro and microscopically examined [41 – 43].

Statistical Analysis

It was analysed with Kruskal-Wallis test whether there were differences between genera, and with Mann-Whitney U test the differences between station locations for each genus. In addition, differences between stations were analysed. SPSS for Windows 11.0 (Chicago, Illinois, USA) software was used for analyses. As a result of the study, values identified as $p<0.05$ were accepted to be significant

RESULTS AND DISCUSSION

In this study 11959 fungal colonies were counted in petri dishes in which air samples were collected from a mine located in Manisa, Turkey. Highest values were seen on 160 Manual 3 (5600 cfu/m^3), Main Desandre 1 (5120 cfu/m^3) and 160 Manual 4 (4200 cfu/m^3) while the lowest values were seen on the outer areas of the mine such as Entrance of administrative personnel building (50 cfu/m^3), air well (38 no'lu istasyon) (80 cfu/m^3) and the managers' office (90 cfu/m^3) respectively. The lowest values inside the coal mine were in 190 Manual 4 (1170 cfu/m^3), B12 Manual Base 2 (1240 cfu/m^3) and B12 Manual Base 3 (1410 cfu/m^3) (Table 2).

Table 2. Distribution of genera in Sampling Points (cfu/m^3)

Genera	1	2	3	4	5	6	7	8	9	10	11	Total
Samplin Points												
1	20		1170	60			70	10			80	**1410**
2			1140	70	20	30	170				60	**1490**
3	80		1980				290	20	70	10	30	**2480**
4	50	10	2750	40	10					70	150	**3080**
5		10	2960	70			200				270	**3510**
6			2620	70		30	680	10		90	100	**3600**
7	50		2370	120		10	750		70	70	50	**3490**
8			810	30		20	310					**1170**
9		40	2690	60			900		50	160	120	**4020**
10	30		2940		50		730		60	50	20	**3880**
11			2490			40	1050	10	60	60		**3710**
12			3010				560			40	300	**3910**
13			2630	30	50	10	720	10		50	200	**3700**
14	10	30	2240		40		1210		170	80		**3780**
15		30	2260	10	20	20	550			60	150	**3100**
16	50		1350	40			1550	10				**3000**
17	40	10	2150	80	70	20	480	20	150		80	**3100**
18			1770	120	140	20	730		170	30	100	**3080**
19	20		980				1210		60	30	200	**2500**
20			1750	30	10		1200	10	20	70	250	**3340**
21			1190		30	10	980			40	270	**2520**
22	40		1120				1490			20	170	**2840**
23		10	1070				1020	10	20	30	660	**2820**
24			1980			10	1180	10		70	110	**3360**
25			880				1730		80	60	30	**2780**
26			300		80	20	610		10	80	140	**1240**
27			420	70	30		760			60	70	**1410**
28			3940	10	30		130	10		50	30	**4200**
29			2550	40	10		1300			40	100	**4040**
30	50	20	1300		30	30	3710	30	200	30	200	**5600**
31			20				4190			60		**4270**
32							3950	30		20		**4000**
33			960			60	1470		100	50	20	**2660**
34			700				2060			60	280	**3100**
35			3580	120			1020	10		70	320	**5120**
36			1030		10		2210			30	120	**3400**
37			1040		50		2260	10	100	80	300	**3840**
38	20		50		10							**80**
39	20		540		10		10	10		10	100	**700**
40	10		40	10	20		10				30	**120**
41	10		10	10							60	**90**
42			10				20				20	**50**

1. *Alternaria sp.*2.*Amorphotheca sp*. 3. *Aspergillus sp*. 4. *Cladosporium sp*. 5. *Fusarium sp*. 6. *Mucor sp*. 7. *Penicillium sp*. 8. *Rhizopus sp*. 9. *Scopulariopsis sp*. 10. *Trichoderma sp*. 11. *Mycelia sterilia*

25 species belonging to 10 genera were identified (*Aspergillus* (8 species), *Penicillium* (5 species), *Rhizopus* (2 species), *Cladosporium* (2 species), *Trichoderma* (2 species), *Alternaria* (1 species), *Amorphotheca* (1 species), *Fusarium* (1 species), *Mucor* (1 species) and *Scopulariopsis* (1 species)). The numbers of colonies per genera are given in table 3.

Table 4a and 4b represents the colony counts of species in the stations. The most abundant species is *Aspergillus flavus*, it is followed by *Penicillium commune*, *Mycelia Sterilia*, *Aspergillus fumigatus*, *Penicillium janthinellum* and *Penicillium chrysogenum* respectively.

Kruskal-Wallis test results show that the difference between the genera is statistically significant ($P=0.00<0.01$). the difference between main carriage canals (station 1) and outdoor areas (Station 6) ($p=0.036<0.05$) and the difference between desandre, carriage and main desande canals (station 4) and outdoor areas ($p=0.034<0.05$) are found to be significant (Table 5).

Table 3. Number of colonies counted in petri dishes and their percentages

Genera	Counts	Percentage %
Aspergillus sp.	6479	54.18
Amorphotheca sp.	16	0.13
Penicillium sp.	4347	36.35
Rhizopus sp.	22	0.18
Alternaria sp.	50	0.42
Trichoderma sp.	173	1.45
Cladosporium sp.	109	0.91
Scopulariopsis sp.	139	1.16
Mucor sp.	33	0.28
Fusarium sp.	72	0.60
Mycelia sterilia	519	4.34
TOTAL	11959	

Table 4a. Distribution of species in Sampling Points (cfu/m^3)

Sampling Point No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Total 1
Alternari alternata	20		80	50			50			30				10		50	40		20			**350**
Amorphotheca resinae				10	10				40					30	30		10					**130**
Aspergillus alliaceus		50					50				110					10				10		**230**
Aspergillus flavus	1100	1030	1760	2640	2490	240	2170	720	2160	2240	2080	2710	2230	2040	2180	1150	2050	1470	980	1430	1080	**35950**
Aspergillus foetidus			90			10		30				20		130								**280**
Aspergillus fumigatus	70			50		60	150		120	380	300		400		80	20	100	170		310		**2210**
Aspergillus melleus		60			60			40	110													**270**
Aspergillus niger				60	230	60		20	120	200		160		70		160					110	**1190**
Aspergillus oryzae			70		180				180			30						130				**590**
Aspergillus terreus			60							120		90				10						**280**
Cladosporium herbarum		70		40	70			30	60				20			20	50	120				**480**
Cladosporium oxysporum	60					70	120						10		10	20	30			30		**350**
Fusarium moniliforme		20		10						50			50	40	20		70	140		10	30	**440**
Mucor racemosus		30				30	10	20			40		10		20		20	20			10	**210**
Penicillium chrysogenum	70				140	300	50		120	20	290				140	130		120	120			**1500**
Penicillium commune		170	110			240	150	290	710	580	750	540	620	600	250	1250	300	590	1070	910	670	**9800**
Penicillium griseofulvum						140	110	20		40		10		300	130		180		10			**940**
Penicillium janthinellum			80		50		350		70		10	10	90	310		170		20		290	310	**1760**
Penicillium spinulosum			100		10		90			90			10		30				10			**340**
Rhizopus oryzae						10					10					10				10		**40**
Rhizopus stolonifer	10		20										10				20					**60**
Scopulariopsis acremonium			70				70		50	60	60			170			150	170	60	20		**880**
Trichoderma aureoviride				60		30	70		40	30		30		60	10					30	40	**400**
Trichoderma hamatum			10	10		60			120	20	60	10	50	20	50			30	30	40		**510**
Mycelia sterilia	80	60	30	150	270	100	50		120	20		300	200		150		80	100	200	250	270	**2430**
TOTAL	**1410**	**1490**	**2480**	**3080**	**3510**	**3540**	**3490**	**1170**	**4020**	**3880**	**3710**	**3910**	**3700**	**3780**	**3100**	**3000**	**3100**	**3080**	**2500**	**3340**	**2520**	**63810**

Table 4b. Distribution of species in Sampling Points (cfu/m^3)

Sampling Point No	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Total 2	Total
Alternaria alternata	40								50								20	20	10	10		**150**	**500**
Amorphotheca resinae		10							20													**30**	**160**
Aspergillus alliaceus			10												20				40	10		**80**	**310**
Aspergillus flavus	960	1040	1570	880	240	390	3650	2330	1170	20		870	670	3460	930	1010		340				**19530**	**55480**
Aspergillus foetidus	110					30								40								**180**	**460**
Aspergillus fumigatus		30	260		60		290	130	50			90	30	70	60	30	20	180				**1300**	**3510**
Aspergillus melleus	10																	20				**30**	**300**
Aspergillus niger			140					90	60					10								**300**	**1490**
Aspergillus oryzae																						-	**590**
Aspergillus terreus	40								20						20		30				10	**120**	**400**
Cladosporium herbarum						20	10							70						10		**110**	**590**
Cladosporium oxysporum						50		40						50					10			**150**	**500**
Fusarium moniliforme					80	30	30	10	30						10	50	10	10	20			**280**	**720**
Mucor racemosus			10		20				30			60										**120**	**330**
Penicillium chrysogenum	220	30	50	170		40		70	280		20	40	60	40	50	120		10				**1200**	**2700**
Penicillium commune	1040	970	930	1340	560	690	130	1080	3070	4190	3910	1220	1910	970	2040	2060						**26110**	**35910**
Penicillium griseofulvum					50			40				10			30				10		20	**160**	**1100**
Penicillium janthinellum	210	20	120	220		30		110	360		20	130	80	10	90							**1400**	**3160**
Penicillium spinulosum	20		80									70	10			80						**260**	**600**
Rhizopus oryzae									30							10						**40**	**80**
Rhizopus stolonifer		10	10				10				30			10				10				**80**	**140**
Scopulariopsis acremonium		20		80	10				200			100				100						**510**	**1390**
Trichoderma aureoviride	20	10			50	60		20			20	30	60	40	30	30						**370**	**770**
Trichoderma hamatum		20	70	60	30		50	20	30	60		20		30		50		10				**450**	**960**
Mycelia sterilia	170	660	110	30	140	70	30	100	200			20	280	320	120	300		100	30	60	20	**2760**	**5190**
TOTAL	**2840**	**2820**	**3360**	**2780**	**1240**	**1410**	**4200**	**4040**	**5600**	**4270**	**4000**	**2660**	**3100**	**5120**	**3400**	**3840**	**80**	**700**	**120**	**90**	**50**	**55720**	**119530**

Table 5. Kruskal-Wallis Test Results of stations

Variable Station		Mean	SE Mean	Median
Median	1	15,00	2,89	15,00
	2	15,00	4,85	10,00
	3	22,5	11,1	20,0
	4	28,46	8,15	20,00
	5	7,50	2,50	10,00
	6	4,00	2,45	0,00

Table 6 represents the descriptive analyses for each genera and station. According to the descriptive statistics the most abundant species in stations are as follows: *Aspergillus* (2783±119), *Trichoderma* (77.5±27.8) and *Amophotheca* (10±10) are most abundant in ceiling areas (station 3); *Penicillium* (1527±442) is mostly seen in Manual areas (Station 2); *Rhizopus* (7.50±4.79), *Cladosporium* (42.5±15.5) and *Alternaria* (37.5±17.5) are mostly seen in main carriage canals (station 1); *Scopulariopsis* (59.2±19.4), *Fusarium* (30±11.3) and *Mycelia sterillia* (155±31.3) are most seen in desandre, carriage and main desande canals (station 4) and *Mucor* (10±4.8) is most abundant in desandre, carriage and main desande canals and in ceiling areas (3rd and 4th stations).

The differences between stations for *Amorphotheca*, *Mucor* and *Fusarium* were not found to be statistically significant ($p>0.05$). Among other genera, *Aspergillus* and *Penicillium* exhibited notable differences in comparisons between stations ($p<0.05$) (Table 7).

The difference between species was found to be statistically significant as a result of conducted Kruskal-Wallis test ($P=0.00<0.01$).

Since they are the most abundant genera found in this study descriptive statistics for species of *Aspergillus* and *Penicillium* were also calculated. Descriptive statistics conducted for *Aspergillus* species yielded the following results (Table 8). *Aspergillus flavus* was found in higher numbers than the others (1321±149).

Descriptive statistics conducted for *Penicillium* species yielded the following results (Table 9). *Penicillium commune* was found in higher numbers than the others (855±152). The difference between species was found to be statistically significant as a result of conducted Kruskal-Wallis test ($P=0.00<0.01$).

As mentioned before, although there are many studies on airborne microfungi in our country and throughout the world, regarding identification of potential allergic species and determination of their seasonal variance; there is no study where potential pathogenic microfungi in interior and exterior air of mines were identified, other than studies that researched pneumoconiosis (anthracnosis), silicosis, emphysema and chronic bronchitis.

Table 6. Descriptive Stattistics for Genera and Stations

Genera	Station	Mean	SE Mean	Median	*Genera*	Station	Mean	SE Mean	Median	*Genera*	Station	Mean	SE Mean	Median
Aspergillus sp.	1	1760	383	1575	*Amorphotheca sp.*	1	2,5	2,5	0	*Penicillium sp.*	1	132,5	63	120
	2	1514	381	1090		2	2,5	1,79	0		2	1527	442	755
	3	2783	119	2815		3	10	10	0		3	810	106	815
	4	1726	230	1750		4	5,38	3,12	0		4	1282	168	1210
	5	1340	215	1155		5	2,5	2,5	0		5	1168	116	1100
	6	130	103	40		6	0	0	0		6	8	3,74	10
Alternaria sp.	1	37,5	17,5	35	*Rhizopus sp.*	1	7,5	4,79	5	*Trichoderma sp.*	1	20	16,8	5
	2	8,33	5,62	0		2	6,67	3,33	0		2	46,67	8,47	55
	3	7,5	7,5	0		3	2,5	2,5	0		3	77,5	27,8	55
	4	9,23	4,73	0		4	5,38	1,83	0		4	46,92	7,54	50
	5	10	10	0		5	5	2,89	5		5	40	10,8	35
	6	12	3,74	10		6	2	2	0		6	2	2	0
Cladosporium sp.	1	42,5	15,5	50	*Scopulariopsis sp.*	1	17,5	17,5	0	*Mucor sp.*	1	7,5	7,5	0
	2	34,2	11,6	20		2	30	17,5	0		2	9,17	3,58	0
	3	15	15	0		3	42,5	14,4	55		3	10	10	0
	4	33,1	12,5	10		4	59,2	19,4	20		4	10	4,8	0
	5	0	0	0		5	5	5	0		5	5	2,89	5
	6	4	2,45	0		6	0	0	0		6	0	0	0
Fusarium sp.	1	7,5	4,79	5	*Mycelia sterilia*	1	80	25,5	70					
	2	15	7,02	0		2	82,5	24,6	60					
	3	12,5	12,5	0		3	110	68,6	70					
	4	30	11,3	10		4	155,4	31,3	150					
	5	7,5	7,5	0		5	303	124	220					
	6	8	3,74	10		6	42	17,4	30					

Table 7. Mann - Whitney U test results pertaining to station locations of genera

Genera	1	2	3	4	5	6	7	8	9	10	11
Stations											
S1-S2	**0.038***	0.79	0.54	0.66	0.84	0.726	**0.011***	0.67	0.712	0.198	0.714
S1-S3	0.166	0.85	0.083	0.215	0.741	0.85	**0.021***	0.405	0.439	0.146	0.773
S1-S4	0.066	0.939	0.651	0.445	0.377	0.79	**0.003***	0.706	0.269	0.169	0.256
S1-S5	0.166	1	0.309	**0.047***	0.741	0.739	**0.021***	0.752	0.85	0.189	0.043
S1-S6	0.258	0.264	**0.014***	0.095	0.896	0.264	0.133	0.306	0.264	0.306	0.217
S2-S3	0.859	0.594	0.069	0.269	0.727	0.834	0.808	0.657	0.349	0.501	0.854
S2-S4	0.561	0.613	0.48	0.864	0.285	0.829	0.624	0.755	0.255	0.978	0.101
S2-S5	0.859	0.789	0.903	0.06	0.574	0.788	0.544	0.778	0.658	0.625	0.024
S2-S6	0.064	0.347	**0.035***	0.198	1	0.101	**0.002***	0.511	0.157	**0.01***	0.424
S3-S4	0.833	0.761	**0.017***	0.39	0.308	0.79	0.192	0.434	0.906	0.493	0.531
S3-S5	0.85	0.85	**0.021***	0.317	0.85	0.739	0.083	0.495	0.089	0.191	0.248
S3-S6	0.366	0.264	**0.014***	0.884	0.59	0.264	**0.014***	0.866	**0.028***	**0.011***	0.621
S4-S5	0.888	0.939	0.734	0.075	0.209	0.899	0.692	1	0.197	0.529	0.308
S4-S6	0.192	0.255	**0.001***	0.267	0.413	0.118	**0.001***	0.301	**0.049***	**0.008***	0.067
S5-S6	0.366	0.264	**0.014***	0.176	0.59	0.091	**0.014***	0.371	0.264	**0.011***	**0.014***

***p<0.05** 1. *Alternaria sp.*2.*Amorphotheca sp*. 3. *Aspergillus sp*. 4. *Cladosporium sp*. 5. *Fusarium sp*. 6. *Mucor sp*. 7. *Penicillium sp*. 8. *Rhizopus sp.* 9. *Scopulariopsis sp*. 10. *Trichoderma sp*. 11. *Mycelia sterilia*

Table 8. Descriptive statistics of *Aspergillus* species

Species	Mean	SE Mean	Minimum	Maximum
A. alliaceus	7,38	3,16	0	110
A. flavus	1321	149	0	3650
A. foetidus	10,95	4,57	0	130
A. fumigatus	83,6	17,3	0	400
A. melleus	7,14	3,34	0	110
A. niger	35,48	9,69	0	230
A. oryzae	14,05	6,78	0	180
A. terreus	9,52	3,87	0	120

Table 9. Descriptive statistics of *Penicillium* species

Species	Mean	SE Mean	Minimum	Maximum
P. chrysogenum	64,3	13,1	0	300
P. commune	855	152	0	4190
P. griseofulvum	26,19	9,33	0	300
P. janthinellum	75,2	16,9	0	360
P. spinulosum	14,29	4,64	0	100

Several organization have determined certain levels for the total fungus numbers in indoor environments. While World Health Organization (WHO) recommended 150 cfu/m^3 and the Indoor Air Quality Association (IAQA) recommended 300 cfu/m^3 as the limit of total number of indoor fungi [17]; Bush and Portnoy [4] and American Industrial Hygiene Association (AIHA) determined 1000 cfu/m^3 value as the upper limit for indoor air. The Commission of European Communities (CEC) classified total airborne fungus levels that can be cultured and reported 1–499 cfu/m^3 as low, 500–999 cfu/m^3 as medium, ≥1000 cfu/m^3 as high and 2000 cfu/m^3 as an extremely high level. While the numbers of microfungi, obtained from sampling locations at interior and exterior general use areas of Eynez Kömür İşletmeleri in Soma district of Manisa Province, varied between 50 and 120 cfu/m^3 at entrance of administrative personnel building, corridor, management room and mine entrance; such values reached very high amounts of 1170 to 5600 cfu/m^3 at various levels of the mine.

It is well known that fungi are able to grow in many environments depending upon environmental conditions and nutrient availability [46]. Underground mines are quite favorable places for microfungal colonisations with their appropriate temperature and humidity levels, wooden constructions and the surfaces the mines create where they are stored.

In studies about airborne microfungi in indoor and outdoor environments, it is seen that dominant genera are *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus* that which are known to have possible allergenic species [3, 35, 49, 50, 51]. Colonies of *Aspergillus, Penicillium, Trichoderma, Scopulariopsis, Cladosporium, Fusarium, Alternaria, Amorphotheca*, *Mucor* and *Rhizopus* were isolated from the mine in this study.

Identification studies revealed that the dominant microfungus in the minery was *Aspergillus* flavus; which is known to be an aspergillosis agent as well as allergenic. Besides some starins of *A. flavus* also produce aflatoxins and under favorable conditions inhalation of the toxins produced may cause health issues [52 – 53].

Aspergillus fumigatus, which is an opportunistic pathogen and of the causal agents of Cystic Fibrosis (CF) and Allergic Bronchopulmonery Aspergillosis (ABPA) was also isolated, is high numbers in the minery. Being the causative agent of invasive aspergillosis which is a deadly disorder for immune compromised patients [54], *A. fumigatus* possesses a serious risk for miners who are also under the risk of occupational diseases related to inhalation of coal dust such as pneumoconiosis, silicosis and emphysema. *A. fumigatus* also entails the risk of potentially producing toxins like gliotoxin and fumitremorgin.

In addition to having allergenic characteristics, *Penicillium commune*, which is present in high numbers in

mines air, is known to produce cyclopiazonic acid [55, 56] as well as potentially toxic compounds like cyclopaldic acid, cyclopiamine, paliatine and rugulovasins. [56, 57].

Although most of the fungi that we identified, besides abovementioned genera, have the potential to form mycotoxins, there are major deficiencies in information required for complete formation of quantitative risk estimations in terms of the inhalation of mycotoxins. Despite the availability of numerous sources on injection of mycotoxins to animals, there are very few studies on inhalation of mycotoxins. There are also a limited number of epidemiological studies, which research exposure to mycotoxins in indoor air and the health effects of these, and these studies generally do not involve conclusive evidence supporting the relationship in question. While it is known that direct contact with mycotoxins and microfungi producing mycotoxins or high levels of inhalation of microfungi spores have significant effects on animals and health effects on humans; currently available sources cannot provide sufficient evidence that inhalation of indoor air contaminated with microfungi leads to quantifiable health issues [58].

Significant job losses that might occur due to health issues will be prevented, treatment expenses will be decreased and quality of life of employees will be increased by initiating certain improvement activities to the benefit of employees. Activities such as using highly effective filters in ventilation systems, decreasing relative humidity in work environments below 50%, periodically cleaning convenient surfaces for fungal growth with antifungal agents, using special masks during works inside the mine, testing employees for allergies and raising awareness among employees can be given as examples to improvement activities that might be conducted in this context. Thus, significant job losses due to health issues will be prevented, treatment expenses will be decreased and quality of life of employees will be increased.

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