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Fungi in air and on wooden constructions of old historical building

Key words: *microfungi, wooden destruction, mycotoxins, building.*

Summary: In this research the microfungi biodestruction of wooden constructions in the historical building was analyzed. Since the main artifacts in the Museum of Folk Architecture and Life of Ukraine "Pyrogovo" are built of wood, they are extremely vulnerable to the action of biological agents, especially microfungi. The house of village Samary from Volyn region, which is the most ancient artifact in the museum, was examined to identify the fungi spectrum on the wood and in the air. 20 samples of building constructions and 8 air samples have been collected inside and outside the studied house. In total, 11 species and genus (35 specimens) of microfungi were identified from 20 samples of building constructions of the studied house. 25 fungi specimens belonging to 6 species (*Aspergillus niger, Alternaria tenuissima, Chaetomium globosum, Mycelia sterilia, Stachybotrys chartarum, Trichoderma viride*) and 4 fungi which was possible identified only to genus *Acremonium, Fusarium, Mocladium* and *Penicillium* were found on wood walls. The most abundant species of the Samary house on the construction substratum were *Aspergillus niger* and *Alternaria tenuissima,* in the air - *Aspergillus flavus, A. repens, A. niger* and *Alternaria tenuissima.*

1. Introduction

Open-air museums are not only one of the most common ways to preserve the cultural heritage of the people, but there are research institutions that collect monuments of material and spiritual culture. Also

skasen is kind of liaison with the people through scientific and educational activities, such as organizing shows, exhibitions, revival of handicrafts, etc. That is why the museum was and remains the real treasure of folk culture and knowledge from antiquity to the present. The current stage of development of the open-air museum is marked quantitative growth and spread throughout Europe. This is largely due to the fact that they have more opportunities in today's information space as covering not only individual monuments with their exposure, but also the spatial environment that can be converted depending on the design decisions of scientists, architects and museum workers [1].

Museum of Folk Architecture and Life of Ukraine "Pyrogovo" is one of the largest open-air museums (skansen) in the world. The museum creation was started in 1969 with funds Ukrainian Society for Protection of Historical and Cultural Monuments and grand opening was held on July 7, 1976. Its architectural ensemble is made up of 6 historical-ethnographic regions of Ukraine: Naddnipryanschina, Slobozhanschina, Polissia, Carpathians, Podillia and South Ukraine, which provide more than 300 architectural exhibits, among them Ukraine's largest collection of windmills and almost 80.000 items life and works of folk art [2, 3]. Skansen expositions reflect the material and spiritual culture of the nation, so preserving the national heritage for future generations is one of the priority directions of the development of any country.

Since the main artifacts in the museum are built of wood, they are extremely vulnerable to the action of biological agents. Factors affecting the operation of architectural monuments may be divided into: abiotic (temperature fluctuations, rain, UV radiation, wind load, etc.) and biotic (fungi, insects, algae, bacteria, etc.). Mycological (fungi) and

entomological (insect) damage of wooden structures are the most common among biological effects and require considerable expenses for the restoration [4-6]. Considering that the museum "Pyrogovo", like most open-air museum, is also a recreational zone, so a constant monitoring will ensure not only the preservation of cultural heritage, but also ecological safety of the area.

1.1. Mycodestruction

Biodegradation is a special type of corrosion-aggressive impact of the environment, based on the influence of living organisms and their metabolic products in the material. Microorganisms that get the surface structures such as fungi (mycodestruction) form enzymes in the life activity that in the interaction with cellulose materials transform their constituents in more available organic compounds (water and carbon dioxide). Usually the end result of this process is the organic matter destruction, i.e. change the anatomical structure of the material and the loss of proper physical and mechanical properties [7-9].

Fungi are always present in the environment as spores and other structures. In the wood fungi get during the growth of trees, timber harvesting, storage, production and operation. However, the damaging effect of fungal agents occurs only under certain conditions. Subsidence of fungal spores in the wood is actually a continuous process, but the intensity can vary seasonal intervals. Spores capable to the surface adhesion and can penetrate by invisible structural breach of wood to deeper layers, while maintaining its viability over decades and start actively functioning in the event of a favorable environment for their development. Although the effect of factors such as the appropriate temperature and

humidity, the lack of light and air flows, the availability of substrate is required for activity of all fungi, but the output from rest takes place only in individual conditions for each species and even strain [10]. In this regard, it is extremely difficult to navigate at the time of occurrence of damage, its root causes and predict the direction and speed of the process.

Damage of the material by microorganisms is a complex process that may be characterized by six stages [11, 12]:

1) transfer microbial spores from the air, water and soil environment;
2) adhesion microbial spores;
3) emergence and growth of micro colonies that are visible with the naked eye and the appearance of corrosive metabolic products;
4) impact of metabolic products on the material;
5) stimulation of corrosive damage related biodegradation;
6) synergy of biodegradation process whose essence is in appearing functional relationships between existing microorganisms on the surface of the material that substantially affect the process of material damage and change its original course [10, 13, 14].

The peculiarity and complexity in the study of cellulose materials is the inability to guarantee that current changes in physical and mechanical properties associated only with the influence of microorganisms. Often, signs of microbial activity on the surface of wooden structures may be due to the influence of abiotic environmental factors (temperature, humidity, etc.) or mechanical stress. In this regard, the model of laboratory experiments with playing a real character of microbiological damage is recommended to confirm the presence and quantitative assessment of the participation of microorganisms in the change process of material properties [10].

The first stage of wood damaging happens with representatives of the department of Ascomycota and anamorphic fungi that use readily available timber components (reserve carbohydrates and other compounds). These fungi destroy the internal contents of cells, substantially without affecting the structure of cell walls, i. e. not decompose lignin cellulose complexes that form the bulk of the timber. Mycological agents of the primary destruction is mostly wood-staining fungi that feed on contents of dead sapwood cells. Settling timber of wood-staining fungi can occur at temperatures from 5 to 30 °C and humidity over 22%. Usually fungi development continues until the wood keeps the natural moisture. After it dries the viability of fungi almost completely stops. The second stage of wood damage is carried out mainly involving bracket fungi and other xylotrophic fungi which may destroy hard-polymer (lignin and cellulose). These fungi are agents of brown and white rot. The final, third stage of wood degradation lasts for decades. It involved a xylotrophic and saprotrophic nutrition. Overall, the rate of wood decomposition depends on various factors: the type of wood, its position in the design, temperature, humidity, and so on [9, 11].

1.2. Effect of mycodestructors on human health

A large number of microscopic fungi (micromycetes) can synthesize mycotoxins and other poisons that have harmful impact on human health, cause deterioration in physiological state and reduce the body's resistance against diseases. Mycotoxins are secondary metabolites, i.e. they appear to have no role in the normal metabolism involving growth of the fungus, and able to initiate allergic or toxic reactions in humans even at low concentrations.

Fungal metabolites mainly fall into the human body in three ways: orally (with an infected food), inhalation (breathing in spores of toxigenic fungi) or by direct contact through the skin [16, 17]. Mycotoxins have four basic kinds of toxicity: acute, chronic, mutagenic and teratogenic [18]. Their toxicity and properties depend on the chemical structure and concentration [17], and the level of production - from the humidity of the substrate and heat conditions. The relationship between the presence of microscopic fungi in the room and the health of residents of buildings generally appear by allergic reactions of varying intensity as rhinitis, asthma, allergic pneumonitis, and various unpleasant and painful health problems largely of unknown etiology such as frequent bronchitis, chronic cough, and irritation [19, 20].

Toxic effects of mycotoxins on health called mycotoxicoses. The symptoms of mycotoxicoses are almost as diverse as the chemical structures of the compounds themselves. Some compounds may elicit few symptoms until death results, while others may produce severe effects including skin necrosis, leucopoenia and immunosuppression. Doses producing chronic disease are usually far below those responsible for acute effects, and so long-term effects such as cancer or tumor induction are undetected at the time of ingestion and, indeed, may remain so until disease is quite advanced [18].

Mycotoxicoses are examples of “poisoning by natural means” and thus are analogous to the pathologies caused by exposure to pesticides or heavy metal residues. The symptoms of a mycotoxicosis depend on the type of mycotoxin; the amount and duration of the exposure; the age, health, and sex of the exposed individual; and many poorly understood synergistic effects involving genetics, dietary status, and interactions with

other toxic insults. Thus, the severity of mycotoxin poisoning can be compounded by factors such as vitamin deficiency, caloric deprivation, alcohol abuse, and infectious disease status [16, 21].

2. Materials and methods

For this research was chosen a wooden house of village Samary, Volyn region. The exhibit was moved to the National Museum Pyrogovo in 1970, but it was built in 1587. This building is the most ancient artifact in the museum and a rare example of traditional polisky construction. The Samary house is a single-chamber building with total area in 24.5 m^2. It has pine wood walls, a straw roof and soil clay floor.

The study was performed in two stages. The first phase consisted of a preliminary visual inspection of the building and describing the monument, which are marked in the mechanical characteristic features (cracks, crumbling, peeling), entomological (tracks of beetles, web) and microbial damage (raids, colonies, stains, changes in color of wood). Initial inspection algorithm also took into account the presence of leaks, dust, humidity and ventilation. The inspection of buildings has been carried out to identify the damage and determine areas of sampling.

After determining locations of sampling, the second phase that consisted of a field (sampling) and laboratory (identification of fungus type) stages was started. Sampling has been performed by the method of selecting fragments of wood, putty, straw or soil. Samples have been taken under the conditions of sterility of the walls, ceiling, floor and door inside and outside the building, on the surface which visually ascertained damage or suitable conditions for its occurrence. Then samples grow in

laboratory. Isolated cultures of fungi have been identified with standard microscopic technics using determination keys [22-25].

For comparison, air samples from outside and inside the building have been taken. The air samples has been selected by sedimentation method: Petri dishes with a dense nutrient environment are left open in the field of research on 15 minutes, then closed dishes are transferred to a thermostat for 24 hours, maintained same time at room temperature and count the number of colonies that grew. Five air samples have been selected inside (at the corners and center of the building) and three – outside the house (placed randomly).

3. Results and Discussion

20 samples of building construction have been collected inside and outside the studied house (Table 1). In total, 11 species and genus (35 specimens) of microfungi were identified from 20 samples of building constructions of the studied house. 20 fungi specimens were found on substratum outside the house and 15 – inside.

Table 1. List of construction samples collected inside and outside the house with identified microfungi

Samples collected inside and outside the house		
№	Description of the sample location	Fungi species or genus identified from this sample
1	Soil floor	*Alternaria tenuissima*

2	Wall 1 with signs of destruction	-
3	Wall 2, wood has stains from leaking and black dots	*Mocladium* sp. 1
4	Ceiling, spot left in the center	*Aspergillus niger, Mocladium* sp. 1
5	Wall 3 with stains from leaking	*Acremonium* sp. 1
6	Wall 4 with a spot of leaking (restored part of the wall)	*Mocladium* sp. 1, *Acremonium* sp. 1, *A. tenuissima, Penicillium* sp. 1, *Mycelia sterilia* (yellow)
7	Ceiling, sample with putrid wood	*Penicillium* sp. 2
8	Wall 1, wood & clay	*Alternaria tenuissima, Alternaria alternata*
9	Ceiling, wood with visible damage	*Fusarium* sp. 1
10	Ceiling, wood with white spots	*A. niger*
	Samples collected outside the house	
№	Description of the sample location	Fungi species or genus identified from this sample
11	Door	*Aspergillus niger*
12	Wall 1 (wood)	*A. niger*
13	Wall 4 (wood)	*A. niger, Chaetomium globosum, Stachybotrys chartarum*
14	Wall 4 (wood)	*A. niger, S. chartarum*

15	Wall 3 (putty)	*A. niger, Alternaria tenuissima*
16	Wall 3 (wood)	*A. niger, Alternaria tenuissima*
17	Wall 2, wood with black spots	*A. niger, Trichoderma viride,*
18	Wall 2 (wood)	*A. niger, Alternaria tenuissima*
19	Roof (inside the thatch)	*A. niger, Alternaria alternata*
20	Roof (outside the thatch)	*A. niger, C. globosum, Mycelia sterilia* (orange)

Source: Own

Despite of the wood destruction from which a sample 2 was taken, it did not bring any result, may be because location near door provide a fresh air inflow, which don't give condition for fungi spores accumulation and species developing. Surprisingly, that a sample 6 from fresh wood wall brought more rich fungi diversity then completely destroyed fragment of old wood. It was expected that destroyed wood construction accumulate fungi spores and structures, but more high diversity of species on fresh wood can be explain by constant water leakage in this place. It is show high importance such factor as humidity for fungi developing.

Only in 3 of 9 samples taken from wooden structures inside the building were found species of fungi, known as destructors of wood. Namely, from a sample 2 (spot of unknown origin on the ceiling) was

identified *Aspergillus niger*, a sample 8 (wood with clay that was be white spots of unknown origin on the ceiling) - *Aspergillus niger*. However, it is not possible to be sure that these species are cause of damages. For confirmation is necessary to study of the damage process in the laboratory. In all 10 samples that were taken outside the house were found well known destructors of wood, such as *Aspergillus niger* (all 10 samples), *Alternaria alternate* (sample 19), *Stachybotrys chartarum* (samples 13, 14), *Chaetomium globosum* (samples 13, 20) and *Trichoderma viride* (sample 17).

25 fungi specimens belonging to 6 species (*Aspergillus niger, Alternaria tenuissima, Chaetomium globosum, Mycelia sterilia, Stachybotrys chartarum, Trichoderma viride*) and 4 fungi which was possible identified only to genus *Acremonium, Fusarium, Mocladium* and *Penicillium* were found on wood walls. Five specimens belonging to four species: *Alternaria alternata, Chaetomium globosum, Mycelia sterilia* and *Aspergillus niger* were found on the thatch. Two specimens belonging to 2 fungi species were found on the wood and clay (*Alternaria alternate* and *A. tenuissima*) and on the putty (*Aspergillus niger, Alternaria tenuissima*). And only one specimen *Alternaria tenuissima* was on the soil floor (Fig. 1).

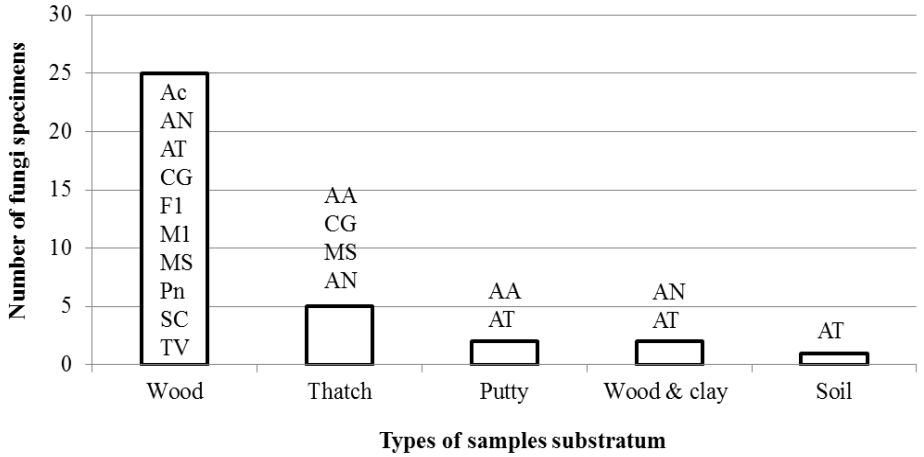

Figure 1. Spreading of fungi specimens on substratum samples (fungi name acronyms as in table 3)

Source: Own

On wall find 23 fungi specimens belonging to 7 species (*Alternaria alternate, Aspergillus niger, Alternaria tenuissima, Chaetomium globosum, Mycelia sterilia, Stachybotrys chartarum, Trichoderma viride*) and 4 fungi which was possible identified only to genus *Acremonium*, *Mocladium* and *Penicillium.* Five specimens belonging were finding on ceiling and roof. In first case it were *Aspergillus niger* and 3 fungi which was possible identified only to genus: *Fusarium, Mocladium* and *Penicillium.* Specimens on roof belong to 4 species - *Alternaria alternate, Aspergillus niger, Chaetomium globosum* and *Mycelia sterilia*. Only by one specimens from one species were identified from the floor (*Alternaria tenuissima*) and door (*Aspergillus niger*) (Fig. 2)

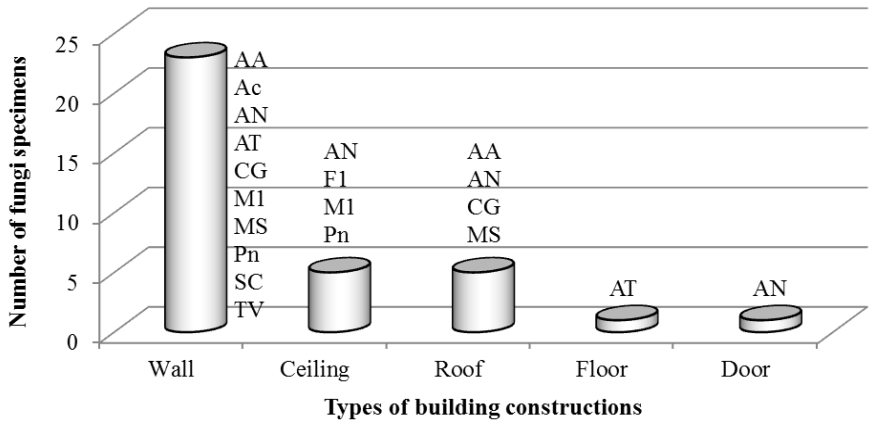

Figure 2. Spreading of fungi specimens on the building constructions of the studied house (fungi name acronyms as in table 3)

Source: Own

From 7 air samples were received 11 species and 5 genera (30 specimens) of microfungi (Table 2). The 8th air sample that was selected outside did not give any results, therefore was not included to the table. 22 fungi specimens belonging to 8 species (*Aspergillus niger, A. flavus, A. repens, A. parasiticus, Alternaria tenuissima, Mycelia sterilia, Absidia spinose* and *A. glauca*) and 5 fungi which were possible identified only to genus *Phoma, Fusarium, Penicillium, Alternaria* and *Trichoderma* were received from air samples taken inside the house. 8 specimens belonging to 7 species (*Rhizopus stolonifer, Aspergillus niger, A. flavus, A. repens, Mucor mucedo, Trichoderma viride* and *Alternaria tenuissima*) and 1 genus *Fusarium* were identified from air samples taken outside the house.

Table 2. List of microfungi identified from air samples inside and outside the house

№ sample	Fungi species or genus identified from this sample	The concentration of spores, CFU/m^3		
		bacteria	fungi	total

Inside the house				
1	*Phoma* sp.	364	332	696
2	*Aspergillus niger, A. flavus, A. repens, Penicillium* sp., *Fusarium* sp., *Alternaria tenuissima, Mycelia sterilia* (orange)	-	844	844
3	*A. flavus, A. repens, Penicillium* sp., *Alternaria* sp., *Trichoderma* sp.	-	1232	1232
4	*A. flavus, A. repens, Mycelia sterilia* (orange), *Alternaria tenuissima*	-	732	732
5	*A. flavus, A. niger, A. parasiticus, Absidia spinose, Absidia glauca*	64	252	316
Outside the house				
1	*Trichoderma viride, Aspergillus niger, A. flavus, Alternaria tenuissima*	120	556	676
2	*Rhizopus stolonifer, A. repens, Fusarium* sp., *Mucor mucedo*	32	292	324

Source: Own

The number of microscopic fungi in the premises is one of the most important indicators of air quality. This index is estimated by the number of colony forming units (CFU) per cubic meter. Analysis of air samples in

the surveyed house indicates that the number of microscopic fungi in certain points of measurements ranged from 252 to 1232 CFU/m^3 inside the house. The highest concentration of spores was obtained from the 3rd sample, which was taken at the center of the research area. Other Petri dishes were placed in the corners, so the results are much smaller, which may explain the more intense air flow due to the gaps in the walls. The concentrations of spores were obtained with only 2 of 3 selected samples outside the house. Their measurement are 292 and 556 CFU/m^3 and have no significant difference.

Regarding the concentration of bacteria spores: insignificant concentration levels were detected in 2 of 5 samples inside the house and in 2 of 3 outside the house. 4 samples may gave no results because of improper conditions for the development of spores that were in the Petri dish. It is also possible that the spores were simply absent in these samples and for more detailed investigation was necessary to use another method of air sampling for microbiological analysis. The role of bacteria in wood degradation is less important because they require a higher water content and have been found mainly in outdoor air samples.

Although airborne bacterial concentrations are usually higher than fungi, in this research there was a different picture. For the indoor air samples, the concentration of total fungi (88%) was higher than the concentration of total bacteria (11%) for all the areas (Fig. 3). For the outdoor air samples, the concentration of total fungi (85%) was also higher than the concentration of total bacteria (15%) (Fig. 4). Also the indoor bacterial concentrations (74%) were significantly higher than the outdoor bacterial concentrations (26%).

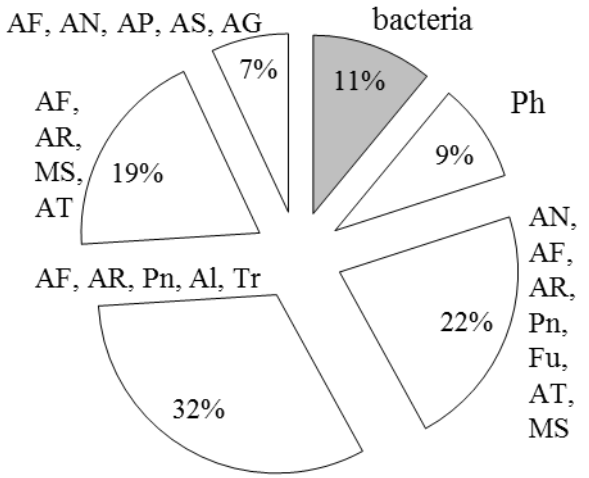

Figure 3. The concentration of spores microfungi and bacteria inside the house (fungi name acronyms as in table 3)

Source: Own

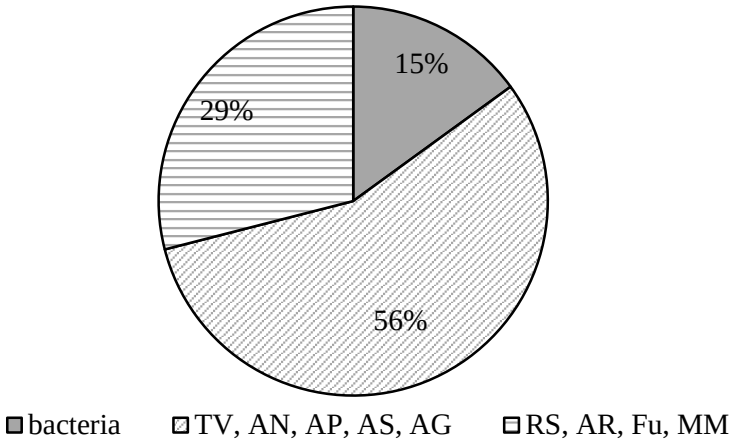

Figure 4. The concentration of spores microfungi and bacteria outside the house (fungi name acronyms as in table 3)

Source: Own

From air and house construction substrates in total 21 species and genus were indentured (Table 3). Common for air and substratum was 6

species (29%), only 10 species (47%) was registered in the air, and 5 species (24%) were found on house constructions. Inside and outside of the house 33% of specimens belonging to 6 species *Alternaria alternata, A. tenuissima, Aspergillus flavus, A. niger, A. repens, Mycelia sterilia* and one fungi which was possible identified only to genus *Fusarium* were registered.

Table 3. List of microfungi identified from construction and air samples inside and outside the building

№	Fungi species or genus	Fungi name acronym	Number of fungi specimens					
			on constructions			in air		
			inside	outside	total	inside	outside	total
1	*Absidia glauca*	AG	-	-	-	1	-	1
2	*A. spinose*	AS	-	-	-	1	-	1
3	*Acremonium sp.*	Ac	2	-	2	-	-	-
4	*Alternaria alternate*	AA	1	1	2	-	-	-
5	*A. tenuissima*	AT	3	3	6	2	1	3
6	*Alternaria* sp.	Al	-	-	-	1	-	1
7	*Aspergillus flavus*	AF	-	-	-	4	1	5
8	*A. niger*	AN	2	10	12	2	1	3
9	*A. parasiticus*	AP	-	-	-	1	-	1
10	*A. repens*	AR	-	-	-	3	1	4
11	*Chaetomium globosum*	CG	-	2	2	-	-	-
12	*Fusarium* sp.	F	1	-	1	1	1	2
13	*Mocladium* sp.	M1	3	-	3	-	-	-
14	*Mucor mucedo*	MM	-	-	-	-	1	1

15	*Mycelia sterilia*	MS	1	1	2	2	-	2
16	*Penicillium* sp.	Pn	2	-	2	2	-	2
17	*Phoma* sp.	Ph	-	-	-	1	-	1
18	*Rhizopus stolonifer*	RS	-	-	-	-	1	1
19	*Stachybotrys chartarum*	SC	-	2	2	-	-	-
20	*Trichoderma* sp.	Tr	-	-	-	1	-	1
21	*Trichoderma viride*	TV	-	1	1	-	1	1
	TOTAL		15	20	35	22	8	30

Source: Own

Inside the house only 43% specimens were accumulated, which are belonging to 3 species *Absidia glauca, A. spinose, Aspergillus parasiticus* and 6 fungi which was possible identified only to genus *Acremonium, Alternaria, Mocladium, Penicillium, Phoma, Trichoderma*. Outside the house only 24% specimens were found, which belong to 5 species *Chaetomium globosum, Mucor mucedo, Rhizopus stolonifer, Stachybotrys chartarum* and *Trichoderma viride.*

The most abundant species on the construction substratum was *Aspergillus niger* and *Alternaria tenuissima,* in the air - *Aspergillus flavus, A. repens, A. niger* and *Alternaria tenuissima*. The spectrum of fungi in building samples was very different from that observed in air. The number of species observed in material samples was low compared to air samples. This may have been partly caused by different laboratory conditions during growing samples. Also it is show that not all spores from air will develop on wood, but need remember that some of this spores can be cause of allergy and other disease for people. Of all the identified fungi can damage the wood following: *Aspergillus niger* causes black mold; *Aspergillus flavus* - cancerogenic mold; *Alternaria alternate* - toxic mold

and blue stains; *Stachybotrys chartarum* - toxic mold; *Trichoderma viride* cases mold and produces enzymes [11].

4. Conclusions

1. In total, 11 species and genus (35 specimens) of microfungi were identified from 20 samples of building constructions of the studied house. 20 fungi specimens were found on substratum outside the house and 15 – inside.
2. 25 fungi specimens belonging to 6 species (*Aspergillus niger, Alternaria tenuissima, Chaetomium globosum, Mycelia sterilia, Stachybotrys chartarum, Trichoderma viride*) and 4 fungi which was possible identified only to genus *Acremonium, Fusarium, Mocladium* and *Penicillium* were found on wood walls.
3. From 7 air samples was received 11 species and 5 genera (30 specimens) of microfungi – 22 specimens inside and 8 outside of the house.
4. The most abundant species on the construction substratum was *Aspergillus niger* and *Alternaria tenuissima,* in the air - *Aspergillus flavus, A. repens, A. niger* and *Alternaria tenuissima*.

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